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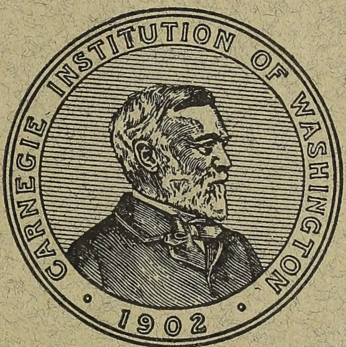

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II. Smith, H. G.—Contribution to the Anatomy and Physiology of *Cassiopea frondosa.* Pages 17-52, 1 plate, 20 text-figures. (July 24, 1936.)

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PAPERS FROM TORTUGAS LABORATORY, VOL. XXXI

I

EFFECT OF TEMPERATURE UPON THE INHERENT POTENTIAL OF VALONIA

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[Issued May 6, 1936]

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EFFECT OF TEMPERATURE UPON THE INHERENT POTENTIAL OF VALONIA

INTRODUCTION

The large potentials normally encountered across the relatively simple protoplasmic layer of the cœnocytic algæ, together with their viability under experimental conditions, make them unique material for the study of bioelectric phenomena. The dependence of the normal potentials of these cells upon factors in their environment has, however, received little attention aside from investigations of the effect of altering the electrolyte composition of the medium (for review of literature, see Osterhout, 1931).

In the interpretation of the physico-chemical nature of the processes giving rise to electromotive forces in living systems, the influence of all environmental factors must be taken into account. Failure so to do has led Osterhout (1931, 1933) to maintain that because potassium salts affect the potential the latter must be *caused* by the diffusion of potassium ions.[1] While "the P.D. of the protoplasm depends on the solution applied to the outside" (Osterhout, 1931, pp. 384-385), this fact does not bestow upon any constituent of the solution the rôle of causative agent. This is beautifully illustrated by the results of Kümmel (1929), who found the potential in *Chara* to be influenced by some seventeen different substances, among them the non-electrolytes sugar and urea. As will appear in this and subsequent papers, temperature, light (Marsh, 1935 b), anesthetics, etc., exert characteristic effects upon the E.M.F. of *Valonia* without necessitating the conclusion that they are primary causes of the potential.

The effect of temperature upon the potential is of prime importance in the determination of the nature of the underlying electrochemical process. The electromotive force of a system in thermodynamic equilibrium (including the diffusion potential) is proportional to the absolute temperature (Q_{10} of 1.04 or less within the biological range of temperatures). As pointed out by Lund (1932a), this rule is not expected to hold for a system in *flux equilibrium* which yields a continuous output of matter and energy, but wherein the E.M.F. is determined by the steady state of concentrations of the electromotively active materials. This type of equilibrium is hypothecated in Lund's theory that continuously maintained bioelectromotive forces are oxidation-reduction potentials intimately associated with the respiratory processes in the living cell (Lund, 1928, 1931; Marsh, 1935a). For the frog's skin (Q_{10} of 2 between 16° and 26° C., Lund and Moorman, 1931) and the Douglas fir (Lund, 1932a, 1932b) the potential follows the expectation for the flux equilibrium, being not proportional to the absolute temperature.

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[1] This argument has recently been negated for *Halicystis ovalis* by Blinks (1935), who found the inherent potential to be independent of potassium or "any concentration gradient existing between the vacuole and exterior of the cell."

In the present paper it will be shown that the normal E.M.F. of *Valonia ventricosa* follows the Q_{10} rule for biological processes generally, so that the potential is not a thermodynamic one as claimed by Osterhout (1931, p. 125). There is also presented a brief analysis of the necessary conditions under which the flux equilibrium will produce a polarity potential following the Q_{10} rule.

METHODS

Cells of *Valonia ventricosa* were impaled on glass capillaries filled with artificial sap (Osterhout, 1931) and supported on glass rings (Blinks, 1930-31). These passed through corks which fitted 250 cc. pyrex beakers. The cells were completely immersed in sea-water.

The beakers were set in a water-bath in which temperature was controlled by adding ice or warm sea-water. The bath was stirred by hand, and its temperature kept under observation. The sea-water within the beakers was not stirred. A thermometer reading to 0.1°C. was placed in the beaker with its bulb at the level of and near to the cell. Readings of this thermometer were recorded as the temperature of the *Valonia.* This assumption was highly probable as judged by the rapidity with which the beakers could be equilibrated with the bath. It was possible in this way to maintain the temperature of the beaker constant to within 1°C. or less.

The water-bath was set on a table about five feet from a window fronting the north sky. Although the magnitude of the potential is in part dependent upon the intensity of illumination (Marsh, 1935b), the *temperature coefficients* herein reported are not due to photosynthesis. Warburg (1919) has shown that photosynthesis in *Chlorella* is independent of temperature when the intensity of illumination falls to around $\frac{1}{70}$ of the value for direct sunlight. The maximum intensity in the present experiments was of the order of magnitude of $\frac{1}{500}$ direct sunlight. The size of the temperature coefficient was not affected by the time of day during which the experiments were performed. Values obtained in early morning, late afternoon, dusk, or at night (illumination by chance reflection from a 2 c.p. lamp lighting the galvanometer scale) did not differ from those obtained at mid-day. The conditions at night are especially convincing, since the intensity of illumination was less than $\frac{1}{10{,}000}$ direct sunlight.

Potentials were measured on a rough potentiometer, consisting of a two-volt lead accumulator with a reversing switch and two Leeds and Northrupp dial resistances in series. One resistance was fixed at 99,990 ohms, the other was used as a variable resistance, R. The unknown potential was connected across the terminals of R together with a tapping key and a Leeds and Northrupp 2320-c galvanometer of 1.3-mm. per microampere sensitivity. The galvanometer was balanced to zero with the variable resistance; a reading lens was used to magnify the excursions of the needle. The E.M.F. was then calculated from the formula $E=\frac{2,000R}{99,990+R}$ millivolts. The maximum current drawn from the accumulator (20 microamperes) was too small to produce an appreciable change in its voltage. R could be varied in 10-ohm steps, hence the working sensitivity of the potentiometer was 0.2 millivolts.

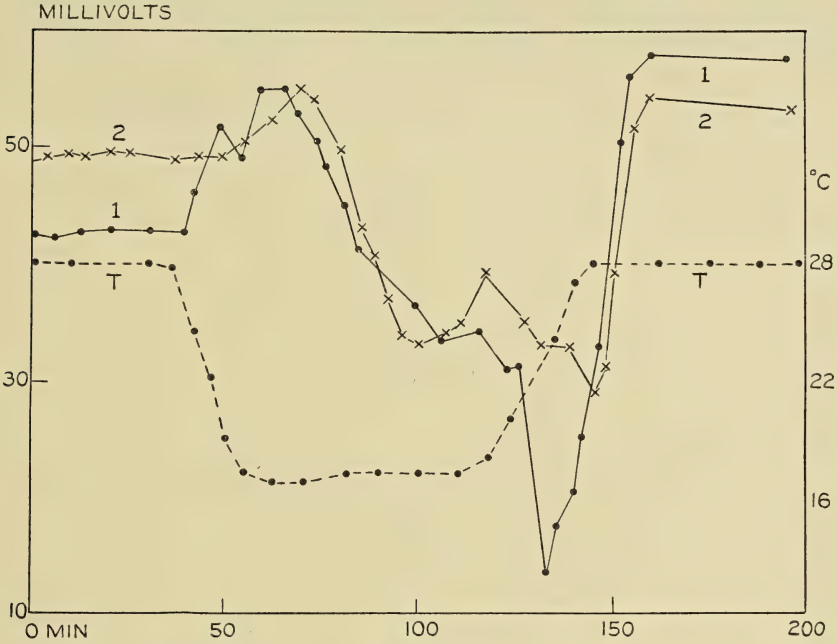

FIG. 1—Time: potential curves for two *Valonia* cells at different temperatures; T, the temperature curve.

Data for curve 1: 28–17.3° 43–32 mv., $Q_{10}=1.32$; 17.3°–28° 32–59.5 mv., $Q_{10}=1.79$.

Curve 2: 28–17.3° 49.5–32 mv., $Q_{10}=1.46$; 17.3–28° 32–54.5 mv., $Q_{10}=1.6$.

The electrodes used were silver wire coated with AgCl at low current density from saturated LiCl solution and soldered to copper leads. It was found advisable to seal these in small glass tubes with DeKhotinsky cement, so that only the coated portion was exposed to the solutions. One electrode made contact with the artificial sap in the capillary, the other with the sea-water in the beaker. The electrodes were practically isoelectric when immersed in the same beaker of sea-water or sap and were satisfactorily stable. A small potential of the order of 1-2 m.v. (sea-water positive) was found when one electrode made contact with artificial sap and the other with sea-water, with a liquid junction between. The electrodes showed no appreciable polarization from the potentiometer current, but were recoated at intervals to insure the maintenance of a continuous AgCl film.

The methods of E.M.F. measurement and temperature control obviously did not attain the highest possible refinement. Nevertheless, it is felt that they were sufficiently precise to establish the values of the Q_{10} reported below. The average potential for twenty-one *Valonia* cells was 32.24 millivolts at room temperature (28 to 30°C.). The changes in potential with temperature were large under the conditions of the experiments. In consequence, the errors inherent in the methods could produce only negligible errors in the values of the Q_{10}, particularly in view of the variability of the latter.

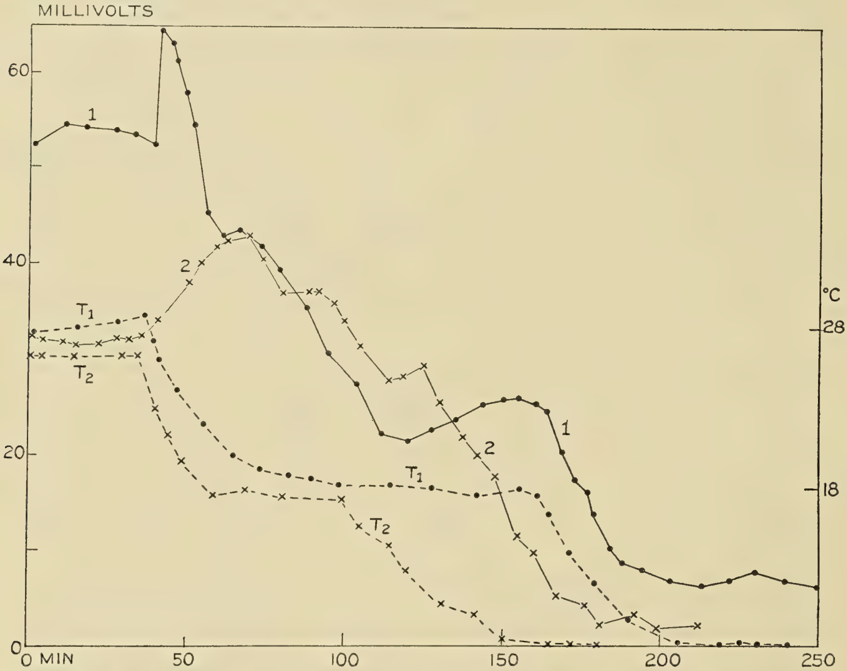

FIG. 2—As in figure 1. T_1 and T_2 correspond to curves 1 and 2. Data for curve 1: 28–18° 54.5–25 mv., $Q_{10}=2.18$; 18–8° 25–6 mv., $Q_{10}=4.2$. Curve 2: 26.2–17.4° 32–37.5 mv., Q_{10} not calculated; 17.4–8° 37.5–2.5 mv., $Q_{10}=15$.

TEMPERATURE COEFFICIENT OF E.M.F.

The variation with time of the E.M.F. of each *Valonia* cell was recorded for two or more temperatures, usually about ten degrees apart (except above 30°C.). The inside of the protoplasmic layer was in all cases positive in the measuring circuit. In the majority of experiments the potential reached an obvious level at the new temperature, as in the curves in figure 1. Where this did not occur an average value of the potential for that temperature was estimated, ignoring the transition portion of the curve. This was the case for curve 1, figure 2, section at 18°, and for the midsection of curve 1, figure 3. In certain cases, to be mentioned below, no estimate of the potential level could be formed. From the pairs of values of the potential so obtained the Q_{10} for the temperature interval was calculated as

$$\left(\frac{E_{t2}}{E_{t1}}\right)^{\frac{10}{t_2-t_1}}$$

where t_2 is the higher temperature in degrees centigrade. The Q_{10} was chosen only as a convenient method of expressing the results. The data do not lend themselves readily to interpretation in terms of any of the theories of temperature coefficients.

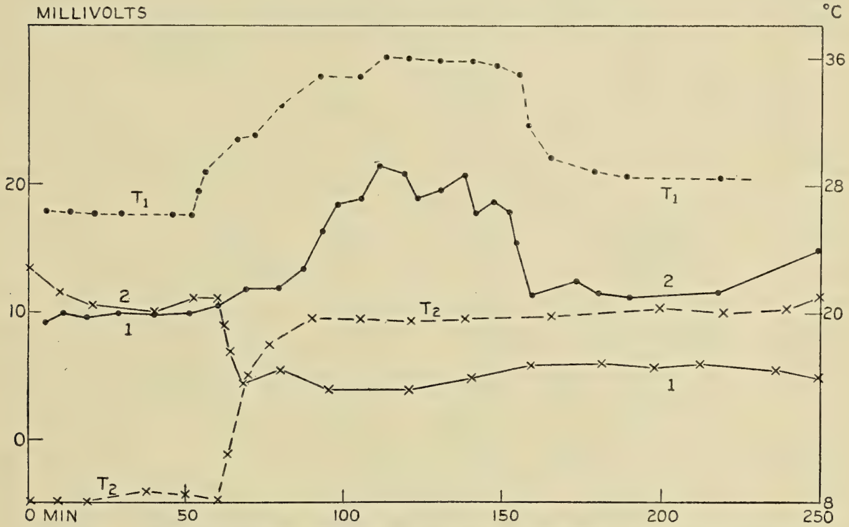

Fig. 3—As in figure 2. Data for curve 1: 26–36° 10–19 mv., $Q_{10}=1.9$; 36–28.4° 19–13.2 mv., $Q_{10}=1.61$. Curve 2 shows undershooting on rising temperature and "anaesthetic" effect of low temperature.

It will be observed in figures 1 and 2 and in curve 2 of figure 3 that changing the temperature brought about a change in potential temporarily opposite to that taken in reaching its final steady level. This phenomenon will be called "undershooting," since it is the reverse of the "overshooting" (Rosene and Lund, 1935) found when oxygen is readmitted to a bioelectric system after a period of absence. The potentials plotted as curve 2 of figure 2 and curve 2 of figure 3 failed to recover from the condition during their stay at the new temperature. In consequence, the Q_{10} values for the temperature intervals could not be calculated.

A total of 69 pairs of values of the potential were obtained on 21 cells, from which 49 values of the Q_{10} were calculated in table 1. Sixteen pairs of values were discarded because of undershooting similar to the above, from which recovery failed to occur during exposure to the new temperature.

Four pairs were discarded because the time course of the potential at all temperatures was so variable as to preclude the estimate of potential levels.

Table 1 presents a summary of the data arranged in four groups. Below each set of values is given the arithmetic mean of the Q_{10} for the temperature interval together with the probable error of the mean. The Q_{10} for the inherent E.M.F. of *Valonia* follows the usual biological rule, being high at low temperatures and decreasing with increase in the temperature level. It will be observed that both the range of variation and the spread of the values for the different temperature intervals become smaller the higher the temperature, as is implied in the decreasing probable errors of the means. The means for the groups 16.4° to 28.4° and 19° to 30° constitute a mutual check upon one another, there being but small difference between the temperature intervals involved. It is of interest to note that the Q_{10} for the former interval, 1.9 ± 0.07, is very close to the values 1.97 and 2.14 found by Lund and Moorman (1931) for the Q_{10} of the E.M.F. of the frog's skin between 16° and 26° C. The mean values for decreasing temperature

TABLE 1

Temperature interval	Direction of change	Q_{10}
6.4–17	Decreasing	3.06
8 –17.2	"	3.82
	"	3.98
8 –17.4	"	1.26
	"	15.0
8 –18	"	4.2
	"	1.3
	Increasing	3.6
8 –19	"	2.34
	"	2.73
6.4–19	Mean	4.15±0.82
16.4–27	Decreasing	2.34
17 –26	Increasing	2.77
17 –27	Decreasing	1.27
17 –28	Increasing	1.55
	"	2.10
17 –28.4	Decreasing	2.16
17.3–28	"	1.32
	"	1.46
	Increasing	1.79
	"	1.60
17.4–26.2	Decreasing	1.43
18 –28	"	2.18
	"	2.23
	Increasing	2.40
	"	1.80
16.4–28.4	Mean	1.90±0.07
19 –29.4	Increasing	1.57
	"	2.12
20 –30	Decreasing	1.63
	"	1.75
20.8–27.2	"	1.70
22.4–28.4	"	1.43
	Increasing	1.83
23 –30	Decreasing	2.18
	"	1.82
23.6–29.2	"	2.19
	"	2.11
	Increasing	1.5
	"	2.06
19 –30	Mean	1.85±0.05
26 –36	Increasing	1.90
27 –32	"	1.22
28.4–32	Decreasing	1.66
	"	1.53
28.4–35.6	Increasing	1.60
28.4–36	Decreasing	1.61
29.4–32	"	1.49
	"	1.67
30 –36	"	1.60
31 –36	Increasing	1.70
	"	1.93
26 –36	Mean	1.62±0.04

do not differ significantly from those for increasing temperature, save in the low temperature group. Figures 1 and 2 and curve 1 of figure 3 show typical experiments from which the Q_{10} values were calculated.

In the low temperature group, the value 15, 17.4 to 8°, is certainly too great. As may be seen in figure 2, curve 2, it is magnified by the failure of the potential to recover from the undershooting induced by the transfer from 26.2° to 17.4°. The values 1.26 and 1.3 in the same group are also obviously divergent, probably for the same reason. In both the latter cases transfer from the higher to the lower temperature produced undershooting. If these three values were omitted, the mean for the low temperature group would be 3.4 ± 0.16. This appears to be the more reasonable value, since the standard deviation and probable error are comparable to those of the higher temperature groups. Since, however, undershooting occurred in some two-thirds of the experiments, omission of values for that reason is a doubtful procedure.

The lowest temperatures employed, 6° to 8°, exerted no injurious effects upon the cells. They did, however, produce a state resembling anesthesia, characterized by a slow recovery upon raising the temperature. An extreme instance is illustrated by curve 2, figure 3. The change in temperature from 8° to 20° caused a drop in potential from 11 to 5 millivolts, where it persisted for two hours beyond the end of the curve as shown in the figure, although the temperature had meanwhile been brought to 26°. When tested again fifteen hours later, the potential had risen to 50 millivolts.

It was found that 36°C. was not injurious for the time limit of the experiments, although the upper limit of temperature tolerance lies not far above this point. Two impaled cells exposed to 38° cytolyzed. Intact cells showed heat coagulation and shrinkage after exposure to 40° for one hour and after exposure to higher temperatures for lesser intervals.

THE UNDERSHOOTING EFFECT

The temporary reversal of direction of change of potential immediately following a change in temperature occurred in about two-thirds of the experiments. Both the occurrence of the effect and the extent of the change in millivolts were related to the rapidity with which the temperature was altered, as is shown in table 2. The first column gives the temperature gradient, the difference between the temperature levels divided by the time in minutes required to pass from one level to the other. The third column records the percentage of experiments in which the undershooting occurred. In the last column is given the average reversal of potential for each temperature gradient calculated as the difference between the extreme point of reversal and the level of potential at the beginning of alteration of temperature. While the number of observations was too small to make the values in the last two columns more than suggestive, the tendency is obviously for greater frequency of occurrence and greater average magnitude of change as the temperature gradient becomes steeper.

The undershooting effect is of more than passing interest. It is a typical characteristic of the time course of transition of the electric polarity of the cell from one flux equilibrium level to another and provides an independent piece of evidence for the polar effect of temperature upon the protoplasmic layer discussed in the following section.

Table 2

Temperature gradient	Number of cases	Per-cent occurrence	Average reversal millivolts
0.0–0.099	7	42.8	8.66
0.1–0.199	12	50	6.5
0.2–0.299	18	61.1	8.1
0.3–0.399	9	100	10.9
0.4–0.499	10	90	10.4
0.5–0.599	6	50	13.0
0.6–0.699	2	100	13.75
0.7–0.799	1	100	3

THE FLUX EQUILIBRIUM AND THE Q_{10}

It has been shown (Marsh, 1935a) that a quantitative relation between the E.M.F. and the velocity of the reactions constituting the respiratory system of the living cell can be derived in terms of the flux equilibrium proposed by Lund. The equations account for the major facts of continuous bioelectric currents and respiratory exchange. The respiratory reactions are assumed to proceed as follows:

$$X \underset{k_2}{\overset{k_1}{\leftrightarrows}} AH_2 + O_2 \xrightarrow{k_3} H_2O + A \xrightarrow{k_4} y \rightarrow CO_2 \qquad \text{(a)}$$

where X is the precursor of the reductant, AH_2. The oxidant, A, of the electromotive material becomes transformed through intermediate stages into CO_2. k_1, etc., are the velocity constants of the respective reactions.

The electromotive force at a locus (phase boundary or plasma membrane) in the living cell may be written:

$$E_h = E_0' - \frac{RT}{2F} \ln P_c \qquad (1)$$

where E_0' is a constant and P_c is the oxygen tension at the locus at flux equilibrium. The difference of potential across the cell will be the algebraic sum of the potentials of two (possibly more) oppositely oriented loci:

$$E_p = -\frac{RT}{2F}\ln\frac{P_{c_1}}{P_{c_2}} = -\frac{RT}{2F}\ln r' \qquad (2)$$

where the subscript 1 indicates the locus of high positive potential in the measuring circuit. The negative sign is conventional only. P_c may be equated to the oxygen tension of the medium, P, as follows:

$$P_c = \frac{P - a + \sqrt{a^2 + P^2 - 2Pb}}{2} \qquad (3)$$

where $$a = \frac{k_1Ck_3 + k_0k_2}{k_0k_3} \text{ and } b = \frac{k_1Ck_3 - k_0k_2}{k_0k_3}$$

k_0 is a diffusion constant for oxygen, C a constant. If (3) is substituted into 2, a and b bear subscripts corresponding to P_c. For complete details the reader is referred to the original paper.

From the above equations, it is obvious that an increase in velocity of respiration due to temperature will simultaneously produce an increase in E_p. This change will be independent of the temperature term in equation (2), since r′ will itself possess a temperature coefficient. Thus the effect of temperature upon a system in flux equilibrium will differ from the effect upon one in thermodynamic equilibrium. No data are available on the temperature coefficient of respiration in *Valonia,* nor in any other biological system showing continuous inherent E.M.F., save for the observations of Lund and Moorman (1931). Nevertheless, in view of the fact that there exists at present no explanation of the simultaneous high Q_{10} for respiration and bioelectromotive force, the writer feels it worth while to outline briefly the possibilities and consequences of such an explanation in terms of the flux equilibrium.

The simplest assumption for purposes of discussion is that all the respiratory reactions at a locus possess similar temperature coefficients. k_0, the diffusion constant for oxygen, will vary with the absolute temperature, or to a negligible degree compared to the velocity constants. The effect of changing temperature upon the values of a and b will then be as if only the constant k_1 were affected. That is, the influence of temperature upon both respiration and E.M.F. can be considered to be due solely to the effect upon the rate of mobilization of the oxidizable material AH_2.

The electromotive system in *Valonia* may be assumed to manifest itself at two loci. The oxygen consumption may then be written $o' = k_0(2P - P_{c1} - P_{c2})$ and the temperature coefficient of oxygen consumption at two temperatures 10°C. apart will be

$$Q_{10} = \frac{(2P - P_{c_1} - P_{c_2})_{t_2}}{(2P - P_{c_1} - P_{c_2})_{t_1}} \qquad (4)$$

where t_2 is the higher temperature. P will, of course, vary with temperature to a negligible degree. The E.M.F. will vary as

$$Q_{10} = \frac{\log r'_{t_2}}{\log r'_{t_1}} \tag{5}$$

If we assume the Q_{10}'s for oxygen consumption and E.M.F. to be equal we may substitute $P_{c1} = r'P_{c2}$ into (4) above and obtain

$$Q_{10} = \frac{2P - [P_{c2}(r' + 1)]_{t_2}}{2P - [P_{c1}(r' + 1)]_{t_1}} \tag{6}$$

r′ for each temperature is determined from (5), provided it is known for any one temperature. At 30°C. the average value of the E.M.F. for *Valonia* was found to be 32 millivolts, so that r′ will have a value of approximately 10. We may take 1.85 to be the value of the Q_{10} of the E.M.F. from 20° to 30° and 3.4 as the value from 10° to 20°. P will lie somewhere between 0.2 and 1 atmosphere due to photosynthesis; the value chosen will not affect the results. If we assign to P_{c1}, the intracellular oxygen tension at the locus of high positive potential, a value very close to that of P at 10°, we may then solve for a and b at each temperature and from the results determine the variation of k_1 at each locus. The actual calculations will be omitted to conserve space.

Under the above conditions it will be found that over the entire interval from 10° to 30°, k_1 at the positive locus will change 100 times, while at the negative locus it will change 3 times. If the Q_{10}'s of oxygen consumption are 1.8 (20° to 30°) and 2.8 (10° to 20°), k_1 varies 50 times over the interval at the positive locus, while at the negative locus it changes 3.5 times. As the Q_{10}'s for oxygen consumption are made smaller, the Q_{10}'s for E.M.F. retaining their observed values, the differential effect of temperature on the two loci disappears. When the Q_{10} for oxygen consumption reaches the values 1.3 and 2.2, k_1 at both loci has these same values of the Q_{10}. (But "undershooting" would not occur; see below.)

With the Q_{10}'s for oxygen consumption and E.M.F. the same, the differential effect of temperature upon the reactions at the two loci decreases as the absolute magnitude of r′ decreases. This is in effect the case for the average value of r′ for a single cell in a tissue like the frog's skin or onion root, where the potentials of a number of cells are summed. We may use the values of the Q_{10} for E.M.F. of *Valonia.* If the average potential of a cell at 30° is 16 millivolts, k_1 changes 25 times at the positive locus and 4.5 times at the negative locus, between 10° and 30°. If the cell potential is 1 millivolt the corresponding values are 16 and 6. The polar effect of temperature decreases with decreasing electropolarity.

It is not possible to obtain a Q_{10} for oxygen consumption greater than that for E.M.F. upon the basis of the assumption of equality of temperature coefficients of all reactions at a locus. This could occur, however, if the individual reaction have different temperature coefficients at the two loci. It would be possible under these conditions for the constant E_0' (equation 1) to vary unequally with temperature at the two loci.

Evidence for the reality of the differential effect of temperature at the two loci is afforded by the undershooting effect following a sudden temperature change described in the preceding section. The effect of an increase in temperature is to increase the rate of formation of the reductant, AH_2; the effect of a decrease in temperature is to decrease the rate. It is evident that the rate of change in concentration of AH_2 between two flux equilibrium levels is greater at the positive than at the negative locus. To illustrate, with rising temperature AH_2 is formed more rapidly at the positive locus, causing a more rapid lowering of P_{c1}. In consequence the polarity potential E_p becomes temporarily smaller. As the negative locus reaches its flux equilibrium level the potential rises, finally reaching a higher steady value at the higher temperature.

This effect could not occur if the acceleration due to temperature were equal at the two loci. At the positive locus P_c is relatively high and AH_2 relatively low as compared to the negative locus. It may be stated as a general principle of the flux equilibrium that, for a given increase in concentration of one of the reacting substances, the rapidity with which a new steady level is sought will be greater the smaller the concentration of the other substance reacted upon. In consequence, if the effect of rising temperature were equal at the two loci, AH_2 would build up more rapidly at the negative locus, and the undershooting would appear in the E.M.F.: time curve as an overshooting. It is necessary, therefore, to assume that the change in velocity of production of AH_2 with temperature is greater at the positive locus. This is in accord with the same deduction arrived at on purely theoretical grounds from a consideration of the conditions of the flux equilibrium which will produce a Q_{10} for E.M.F. and oxygen consumption of comparable magnitudes. The undershooting effect is, thus, a test of the validity of the simplifying assumption that the effect of temperature is principally upon the velocity of production of AH_2, for if the latter is true, undershooting should be observed for any cell or tissue having a Q_{10} for oxygen consumption approximately equal to the Q_{10} for E.M.F., save, possibly, when the average value of r' for the tissue cells is very small. In terms of the flux equilibrium the electropolarity of the living cell is the result of a quantitative polar difference in the velocity characteristics of the chemical system producing the electromotively active materials. Electrical, chemical and physiological polarity are thus only different aspects of the same phenomenon.

While this discussion is of necessity both general and diffuse, it offers a rational basis of interpretation of the large Q_{10} of bioelectric potentials which does not exist outside of the flux equilibrium. It is a fact not explicitly recognized in the literature that any theory which seeks to interpret bioelectric currents in terms of the differences in inorganic ion content of the cell and medium must base its explanation of any change in potential, the medium remaining constant, upon a change in cell permeability (whether conceived of as pore size, solubility, "ion mobility" or "loose chemical combination"). This is an unstable foundation. To the writer's knowledge there is no rational definition of permeability which is of service in this connection, nor is there any theoretical relation between cell permeability and E.M.F.[1] In stimulation an increase in permeability is stated to be associated with a decrease in E.M.F.; in narcosis a decreased permeability accompanies a decreased E.M.F. Temperature and light increase both permeability and E.M.F. Aside from oxygen lack, permeability seems to be independent of oxygen pressure, while the E.M.F. increases with oxygen pressure (up to several atmospheres for the onion root and frog's skin; unpublished results). In the light of the temperature coefficient of E.M.F. reported here, it is interesting to note that the Q_{10} of electrical conductivity of cells and tissues, which is stated to be a measure of permeability (Osterhout, 1922), varies in different materials from 1.05 to 1.48 between 10° and 45°C. (Belehradek, 1935), the values being virtually independent of the temperature level. Finally, to this list of difficulties may be added the futility of attempting to account by means of the permeability concept for the typical E_p: time curves of transition from one environmental state to another (such as undershooting with temperature, overshooting with oxygen, or the partial independence of polarizing currents found for the E.M.F. of the onion root, Marsh, 1930, pp. 459-462). "The word permeability in biology is largely a cloak for ignorance." (Stiles, 1924, p. 1.) When resorted to as an explanation of change in bioelectric currents it is literally a Maxwell's demon.

GENERAL CONCLUSIONS

The E.M.F. found across the protoplasmic layer of *Valonia* is not a thermodynamic one. It is produced by an oxidation-reduction system in flux equilibrium at phase boundaries within the cell. The E.M.F. is not primarily determined by the external medium. The influence of the salt content of the sea-water upon the inherent potential is fundamentally no different from the influence of the composition of the medium upon any other biological process, as respiration, irritability, contractility, etc., where specific electrolytes in different proportions condition, but *do not cause,* the process.

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[1] For literature see Stiles (1924) and Gellhorn (1929).

SUMMARY

1. The effect of temperature upon the inherent potential of impaled cells of *Valonia ventricosa* was determined between 6° and 36°C.

2. The Q_{10} of the E.M.F. was found to have the following mean values:

(6.4-19°) 4.15 ± 0.82; (16.4-28.4°) 1.9 ± 0.07;
(19-30°) 1.85 ± 0.05; (26-36°) 1.62 ± 0.04.

3. The extremes of the temperature range caused no injury to the cells. The lowest temperatures exerted an "anesthetic" effect.

4. During transition from one temperature level to another, the E.M.F. underwent reversal of direction of alteration, or undershooting, whose occurrence and magnitude were dependent upon the rate of change of temperature.

5. The high Q_{10} of E.M.F. is shown to be explicable in terms of the flux equilibrium concept of the respiratory process which forms the basis of Lund's oxidation-reduction theory of bioelectric potentials. The phenomenon of undershooting is similarly explained.

6. An analysis of the conditions under which the flux equilibrium will produce a high Q_{10} for inherent cellular E.M.F. shows that a polar difference in the velocity characteristics of the electrochemical system must exist within the cell boundaries. The implications of the undershooting effect independently testify to the same conclusion.

7. It is concluded that the inherent E.M.F. of *Valonia* is not a thermodynamic one, but is produced by an oxidation-reduction system in flux equilibrium.

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PAPERS FROM TORTUGAS LABORATORY, VOL. XXXI

II

CONTRIBUTION TO THE ANATOMY AND PHYSIOLOGY OF CASSIOPEA FRONDOSA

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[Issued July 24, 1936]

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CONTRIBUTION TO THE ANATOMY AND PHYSIOLOGY OF CASSIOPEA FRONDOSA

INTRODUCTION

This research was undertaken, in the first place, to extend our previous scanty knowledge on the physiology of feeding and digestion in the Scyphozoa. *Cassiopea* was selected as the experimental material for two reasons, it is a member of the Rhizostomeæ in which the mode of feeding is particularly interesting owing to the sub-division of the mouth, while certain species, including the one studied, possess zooxanthellæ. Finally, other aspects of the structure and physiology of species of this genus have been extensively studied, notably at the Tortugas Laboratory, by Mayor and others.

It was originally intended to work on *C. xamachana*, which was very abundant at one time in the moat at Fort Jefferson. Recent changes in the conditions in the moat, the result of silting up, have caused the complete disappearance of the species from this locality. In the absence of this species, *C. frondosa* was investigated and this, although less hardy than *C. xamachana*, proved satisfactory material.

Thanks are due to Professor C. M. Yonge at whose suggestion and under whose direction the work was undertaken, to Dr. W. H. Longley for his generous help and hospitality while working at the Tortugas Laboratory, and to Dr. L. H. N. Cooper for advice on methods of estimating the phosphate and nitrate content of sea-water. Acknowledgments are also due to the Royal Society of London and to the Carnegie Institution of Washington for financial assistance.

MATERIAL AND METHODS

C. frondosa was obtained from a soft sandy shoal to the east of Fort Jefferson in water about 25 feet deep. With the aid of a water-telescope the animals could be seen from the surface lazily pulsating at the bottom, but as the sand was easily stirred up and obscured vision the medusæ were collected by means of the diving helmet. They were placed in tanks in the laboratory but could not be kept healthy for more than a fortnight under ordinary aquarium conditions.

The feeding habits and digestive enzymes were studied over a period of 8 weeks and the methods adopted will be more conveniently described later. To determine the site of absorption and excretion, animals were fed with fish blood or injected with a suspension of carmine and fixed in Bouin or Flemming at varying intervals. Material for histological work was also fixed in Bouin or Flemming. Formalin, 5 per cent in sea-water, was a very useful fixative as it caused less shrinkage of tissues. Subsequent sectioning was done in the University Department of Zoology at Bristol and Aberdeen. The stains used were Delafield's hæmatoxylin and eosin, Heidenhain's hæmatoxylin, Mallory's triple stain, a combination of safranin and light green, hæm-alum and eosin, and azo-carmine.

A carmine-gelatin mass injected into the cœlenteron of living medusæ helped in the elucidation of the canal system. Tissue was macerated by Hertwig's method and then stained with Beale's carmine. For estimation of phosphates the cœruleomolybdic method of Denigès (Atkins, 1923), was used and for estimation of nitrates, Harvey's (1928) reduced strychnine method, the reagent having been prepared previously at Bristol.

ANATOMY AND HISTOLOGY

Cassiopea frondosa is a Scyphomedusan belonging to the Rhizostomeæ and found, according to Mayor (1910), throughout the West Indian Region and the Florida Reefs, although one specimen identified by Rao (1931) as *C. frondosa* has been found near Maskat, at the head of the Arabian Sea. It prefers a clear sandy bottom and lies, with oral arms uppermost, pulsating sluggishly. Bigelow (1893) found many of these medusæ in a lagoon at Bimini, in the Bahamas, and Mayor (1910) adds that they are commonest in lagoons surrounded by mangroves. The Marquesas Keys, about 40 miles east of Loggerhead Key, enclose such a lagoon, but not a single specimen of either *C. frondosa* or *C. xamachana* could be found there. The nature of the bottom, soft slimy mud, probably accounts for their absence. The largest specimen so far recorded was found by Mayor near Port Royal, Jamaica, the bell measuring 259 mm. in diameter, and he further states that the medusæ in Jamaica attain a far greater size than in Florida, most of them measuring over 120 mm. in diameter. The majority of the specimens obtained at the Tortugas were between 50 and 100 mm. in diameter while the largest specimen measured 240 mm.

EXTERNAL APPEARANCE

The external appearance has been so well described by Perkins (1908) and Mayor (1910) that apart from a few extensions a brief summary will suffice. The general color varies from light or greenish brown to blue with variable white spots on the bell. There are always 12 marginal sense-organs, 4 perradial, 8 adradial, and no ocelli. The marginal lappets number 60, 5 between each pair of marginal sense-organs and those on either side of the sense organs are half the width of the other lappets. Unlike

C. xamachana the center of the ex-umbrella is not concave or only very slightly so. Perkins (1908) states that a raised ring is present on the ex-umbrella of *C. xamachana* but not in *C. frondosa.* However, a raised ring was found on the ex-umbrella of every specimen of *C. frondosa* obtained for this work. Four pairs of mouth-arms arise from the oral discs and each gives off a number of short lateral branches. The oral mouths are frilled structures situated on the distal side of the arms from the sub-umbrella. When the animal is not feeding, these mouths remain closed (fig. 1); when feeding they open widely (fig. 10). Associated with the oral mouths are the opaque, white oral vesicles numbering about 40 to each arm. Perkins also states that the oral arms are shorter than in *C. xamachana* and do not extend to the margin, but this can not be accepted as a diagnostic feature because, as shown in Plate I, the arms may extend beyond the margin. Four small sub-genital pits also occur interradially beneath the gonads. Bigelow (1893) found both sexes and was able to distinguish between males and females. In the latter the oral vesicles are crowded at the oral disc and are larger than those on the arms. No oral mouths occur in that region. Eggs are attached to the vesicles of the disc and remain there until they reach the stage of ciliated planulæ. In the male the vesicles are not crowded at the center and oral mouths are present. Similar conditions have been described in *Mastigias papua* by Uchida (1926, a).

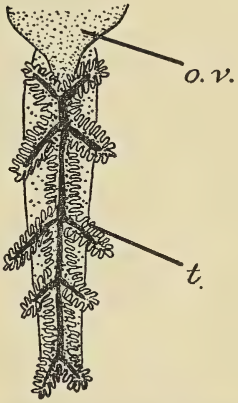

FIG. 1—Oral mouth closed. o.v., oral vesicle; t., tentacle.

CANAL SYSTEM

The oral mouths communicate with the canals of the arms, the short lateral arm canals passing into the main one of each arm (fig. 2, a.c.). Each pair of the 8 large arm canals fuse as they approach the oral disc so that only 4 canals enter the disc. These are continued to the center where they meet to form an irregular cross. Between each successive pair of gonads is a small bilobed opening, the cœlenteric opening of the axial canal which communicates with the common arm canal (fig. 9, ax.). From the cœlenteric cavity 24 radial canals, situated just below the ectoderm of the sub-umbrella, pass out to the margin, 12 of them going to the marginal sense-organs. Between the radial canals are numerous anastomosing canals which unite with the radial canals to form a continuous network. The canal systems of the Rhizostomeæ have been worked out in detail by Stiasny (1921, 1923).

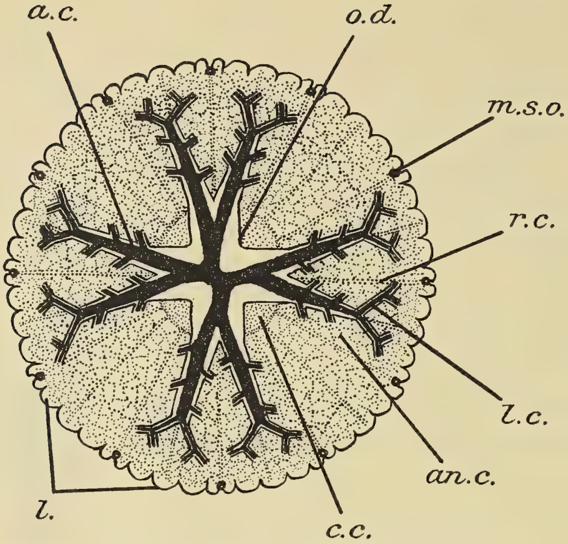

FIG. 2—Diagram of canal system. a.c., arm canal; an.c., anastomosing canals; c.c., cœlenteron; l., lappet; l.c., canal of lateral arm; m.s.o., marginal sense organ; o.d., oral disc; r.c., radial canal.

UMBRELLA

The greater bulk of the umbrella consists of a thick, rigid, gelatinous substance, the mesoglœa, which is covered on the external surface by ectodermal epithelium. Internally it is traversed by the radial canals and a network of smaller canals. Figure 3 shows a section of the marginal part of the umbrella passing through a marginal sense-organ (m.s.o.) and a radial canal (r.c.). The radial canal is lined with flattened endodermal

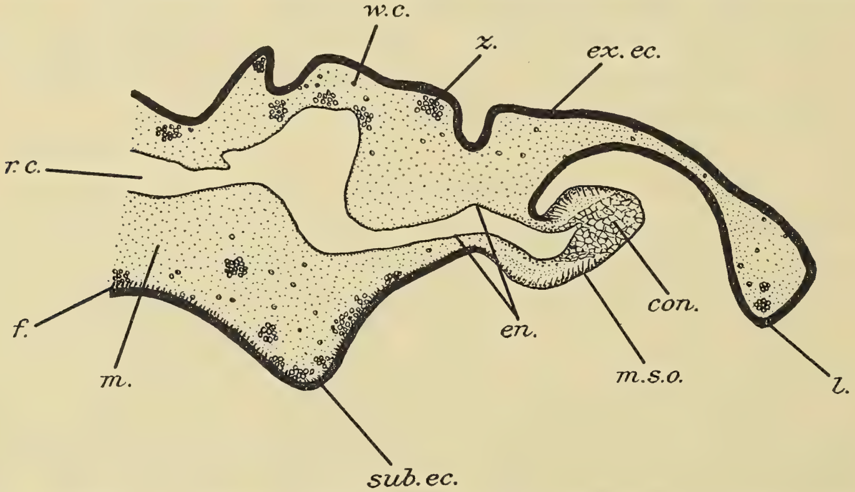

FIG. 3—Section of umbrella passing through a marginal sense-organ. x 78. con., concretionary crystals; en., endoderm; ex.ec., ex-umbrella ectoderm; f., muscle fibers; m., mesoglœa; sub. ec., sub-umbrella ectoderm; w.c., wandering cell; z., zooxanthellæ. Other lettering as before.

cells (en.) and extends up to the sense-organ. As the endodermal cells approach the sense-organ they become larger and pass imperceptibly into a mass (con.) occupying the cavity of the sense-organ and stated by Mayor (1908) in *C. xamachana* to be crystals of calcium oxalate. The ectodermal cells in the region of the sense-organ are elongate and are separated from the concretionary crystals by a granular mass, probably secreted by the ectoderm.

The ectodermal cells of the ex-umbrella are columnar in shape, about 10 μ tall and with large nuclei situated at the bases of the cells (fig. 4, A). A few nematocysts (nem.) occur between the cells and also some dark-staining mucous masses (m.c.), but the majority of the cells are vacuolated, due, perhaps, to the discharge of their secretion. About the distance of one-third of a radius from the margin, the ectoderm becomes very convoluted and is thrown into ridges to form a concentric ring (fig. 13, ex. r.). Here, the cells are larger and have numerous nuclei at the base (fig. 4, B). The cell boundaries are not distinct in the region of the nuclei and the outer portions of the cells are very vacuolated, with here and there mucous

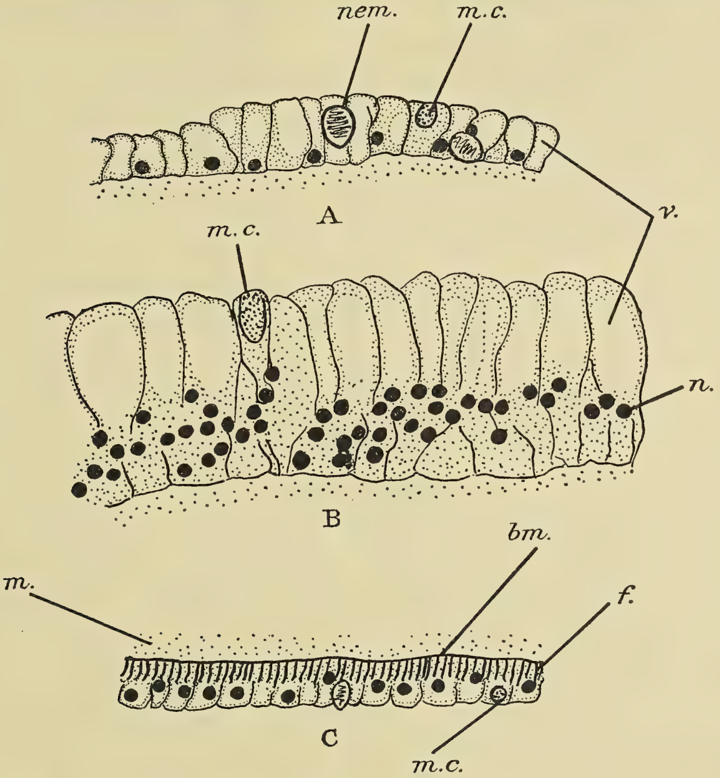

FIG. 4—Ectodermal epithelium. x 934. A, from the ex-umbrella. B, from the ring on the ex-umbrella. C, from the sub-umbrella. b.m., basal membrane; m.c., mucous cell; n., nucleus; nem., nematocyst; v. vacuole. Other lettering as before.

masses. Toward the margin the ectodermal cells become smaller and flattened and are continued over the edge of the lappet into the sub-umbrella ectoderm. The ectodermal cells of the sub-umbrella are only 3.5 μ high and the nuclei lie nearer the center of the cells (fig. 4, C). A few contain mucus but most of the cells are vacuolated. Nematocysts occur occasionally. Underlying the sub-umbrella ectoderm there is a layer of muscle fibers (f.) supported by a basal membrane (b.m.). There are no muscles connected with the ex-umbrella. The ectoderm of the sub-umbrella also lines the sub-genital chamber, and underneath the gonad the cells become very thin and flattened and are closely applied to the sub-umbrella endoderm to form a delicate membrane.

The mesoglœa consists of a rigid, hyaline matrix with, as in *C. xamachana* (Bigelow, 1900), 3 kinds of cells. (1) Small star-shaped cells measuring about 5 μ in diameter with long processes or fibers passing from them through the mesoglœa, which they may help to strengthen. (2) Larger, wandering cells, about 10 μ in diameter, irregular in outline and with large vesicular nuclei. Uchida (1926, b) has found that, in a number of genera of the Rhizostomeæ, the white spots on the umbrella are formed by crowds of modified wandering cells which are "cartilaginous" in nature, thus imparting a firmer consistency to the mesoglœa. These cells were not observed in *C. frondosa*. (3) The zooxanthellæ, the structure and distribution of which will be described later.

ORAL ARMS

The sub-umbrella ectoderem is continued over the oral arms to the tentacles surrounding the oral mouths. Each tentacle consists of a single layer of cells with numerous nematocysts surrounding a core of mesoglœa. Granular endodermal cells line the canals and these are 5 μ high with numerous mucous cells and nematocysts. Associated with the oral arms are the oral vesicles (fig. 5, A), oval and hollow appendages, their cavities communicating with the canals of the arms. They consist of ectoderm and endoderm separated by a layer of mesoglœa. The oral vesicles possess very many large nematocysts in the ectoderm, usually in clusters or batteries which give the vesicle a warty appearance. A number of the larger vesicles on the arms were seen to contain greyish bodies (g.b.) in the lumen, and when examined under the microscope these were found to be little bags of nematocysts and mucous cells. In sections, similar bodies were seen in the mesoglœa of the arm at the base of the oral vesicle and it is possible that they are then passed into the canal of the arm and thence to the cavity of the vesicle. No nematocysts were seen in the mesoglœa of the vesicle. The nematocysts of the vesicles are very large, about 20 μ long. When treated with acetic acid the filaments are shot out. Considering the size of the nematocysts, the filaments are surprisingly long, from 2 to 3 mm.

When stimulated by the presence of small crustaceans, apertures appear at the tips of the oral vesicles and the bags of nematocysts are shot out at the prey (fig. 5, B).

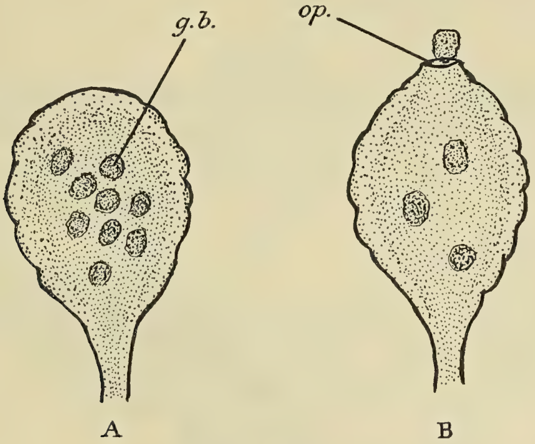

FIG. 5—Oral vesicles. A, at rest. B, ejecting masses of nematocysts. g.b., nematocyst mass; op., opening at tip.

CŒLENTERON

Between each pair of bilobed openings of the axial canals there is a triangular area formed by a very much convoluted layer of endodermal cells, the plaited membrane (fig. 11, p.m.). This is separated from the sub-umbrella ectoderm and endoderm by a layer of mesoglœa and near the apex of the triangle are the gastric filaments (g.f.). In the mature medusa the gonad develops in the substance of the mesoglœa between the plaited membrane and sub-umbrella endoderm and distal to the area bearing the gastric filaments (fig. 6). The sub-umbrella endoderm forms a double layer, the inner being the germ-layer (g.l.), and terminates before it reaches the area bearing the gastric filaments, *i.e.* it at A, (fig. 6).

In the plaited membrane the endodermal cells are granular, about 10 μ tall, with very distinct cell boundaries, except in the region of the gastric filaments, where they become irregular. Nematocysts and mucous cells are numerous. From the endodermal cells arise the gastric filaments which form a broad band across the plaited membrane. Each is cylindrical and formed of a single layer of cells surrounding central mesoglœa. Mucous cells occur in large numbers and also nematocysts, especially near the tip of the filament. The characteristic feature is the presence of large, pear-shaped gland cells which form the greater part of the filament (fig. 17, g.c.). These cells are 10 μ high with large nuclei situated at the narrower, inner ends and possess rounded masses of secretion. In structure they are similar to the granular gland cells described by Yonge (1931, a) for the Madreporaria. Between the gland cells are interstitial cells which consist of wandering cells, mucous cells and nematocysts. The central mesoglœa often contains wandering cells and zooxanthellæ. There is no evidence of the flattened endodermal cells covering the gastric filaments described by Uchida (1926, a) in *Mastigias papua.*

GONADS

As stated earlier, Bigelow found separate sexes in *C. frondosa,* and this has been confirmed. Over a hundred specimens were examined, and it was found that males and females occur in approximately equal numbers. On the other hand, Perkins (1908) working on *C. xamachana* could not be certain of the sex of the animals—whether hermaphrodite or diœcious. It seems improbable, however, that two such closely related species should differ to this extent.

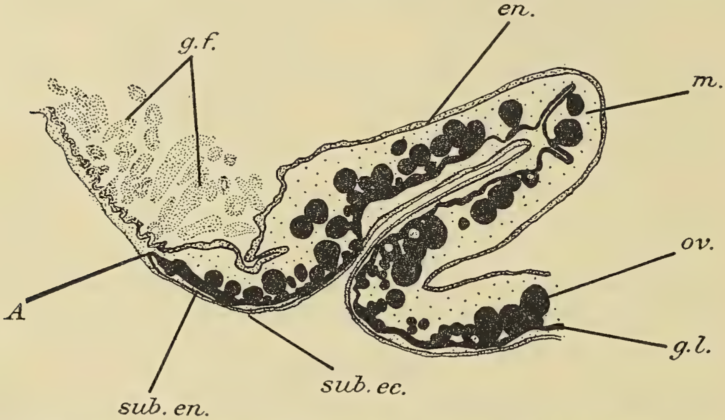

FIG. 6.—Section passing through gonad and gastric filaments. x 48. g.g., gastric filaments; g.l., germ layer; ov., ovum; sub.en., sub-umbrella endoderm. Other lettering as before. A, marks region where sub-umbrella endoderm bends back to form germ layer.

There are 4 gonads, although one medusa was found possessing only 3 gonads. These are formed from the layer of reproductive cells which is, in turn, an extension of the sub-umbrella endoderm. In the female the ova (ov.) are budded off and lie free in the mesoglœa (figs. 6, 7). The ovum is very granular and has a large reticular nucleus with a conspicuous karyosome. In the male the reproductive cells multiply to form vesicles (ves.) which become invaginated at the end nearest the plaited membrane. At the invaginated end the cells increase and pass into the cavity of the vesicle (fig. 8). Passing from the invaginated end to the opposite side of the vesicle are the spermatocytes (s_1), spermatids (s_2) and finally the spermatozoa (s_3) which are arranged in groups with the tails radiating outward. Faintly staining masses occur as in *M. papua,* which Uchida (1926, a) suggests are the remains of disintegrated nutritive cells.

The eggs in *C. frondosa* are discharged into the cœlenteric cavity and segmentation may begin there. They then pass out by way of the axial canal to the oral disc where they remain until they are planulæ, still enclosed by egg-membranes and cemented in masses to the bases of the vesicles. The discharge of the eggs is brought about by distension of the plaited membrane which finally ruptures. Stiasny (1921) suggests that the eggs may pass to the exterior by way of the sub-genital pit. Actually this was observed, but it is believed to be the result of accidental damage to the sub-umbrella tissue covering the gonad. Before the eggs can pass to the

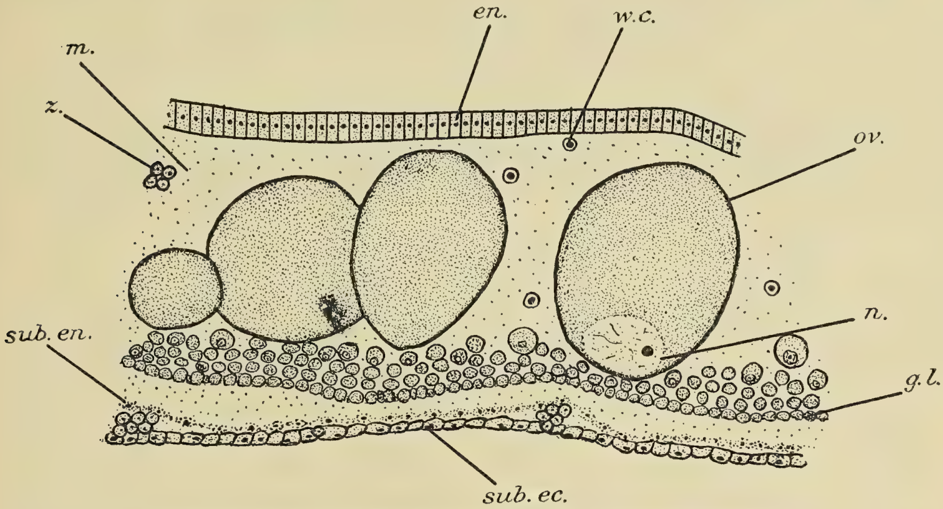

FIG. 7—Section through ovary. x 300. Lettering as before.

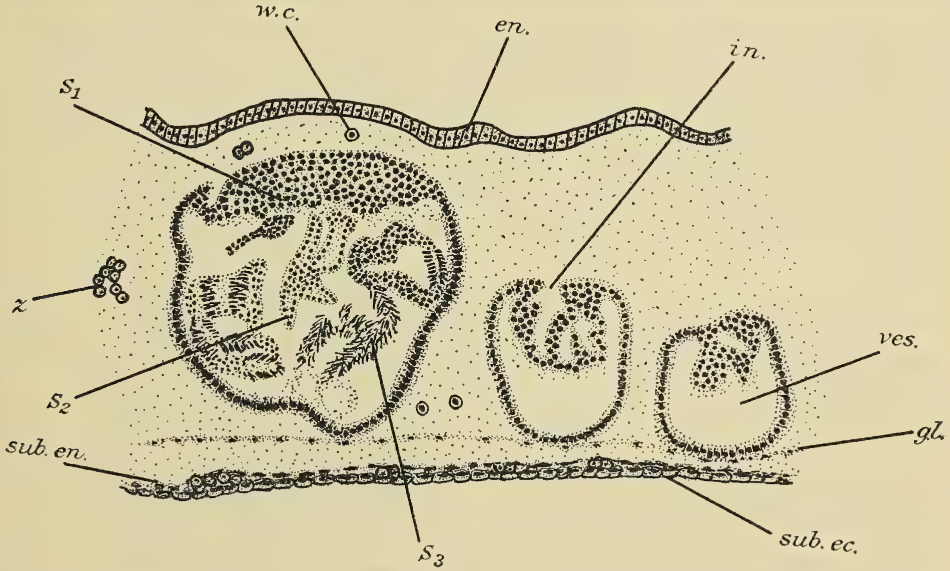

FIG. 8—Section through testis. x 214. in., invagination; s_1, spermatocytes; s_2, spermatids; s_3 spermatozoa; ves., vesicle. Other lettering as before.

sub-genital chamber the important germ layer would have to be ruptured, which seems very improbable.

FOOD AND FEEDING MECHANISM

FOOD

A study of the reactions to various types of food showed that *C. frondosa* is a specialized carnivore. Crustaceans from the plankton were taken eagerly when fed to the jellyfish, and also small pieces of mollusc flesh and fish blood.

CAPTURE OF FOOD

The proximity of food acts as a stimulus notably to the oral vesicles which bend from side to side and become attenuated. A opening appears at the tips of the oral vesicles (fig. 5, B) and small grey bodies, found to be bags of nematocysts and mucous cells, are shot out in strings of mucus at any crustacean that happens to come within range. An animal when caught struggles for a short time but death or paralysis takes place very quickly and it is drawn down by the mucous strings on to the oral mouth which opens to receive it. Bigelow (1893) also observed crustaceans caught by the oral vesicles and compares the mechanism to a trap whereby the vesicle, having forced the prey into the oral mouth, forms a lid over the mouth to prevent its escape. This has not been confirmed. The oral vesicle may bend over and help to place the prey into the oral mouth, but as soon as it has done so it rights itself. Food may also be captured by the tentacles without the aid of the vesicles. If food is placed on a tentacle, neighboring tentacles bend over to help it and with a combined effort the food is thrust into the mouth and is carried by cilia to the cœlenteron. Muscular action is involved in the movements of the oral vesicles and tentacles and in the opening and closing of the oral mouths. Once inside the oral mouth, conveyance of food is effected entirely by cilia.

CILIARY CURRENTS

The oral mouths open only when there is a food stimulus, and it was necessary to feed the jellyfish before adding fine sand particles or a suspension of carmine to determine the internal ciliary currents.

INTERNAL CURRENTS

In the canal of the oral arm two tracts of cilia were distinguished, one on the floor and the other on the roof. The former is continuous with the floor of the oral mouths and the cilia produce an outgoing current, while on the latter the cilia produce an ingoing current. In figures 9 and 12, A, these tracts in the arm canal are indicated by arrows, feathered arrows representing the ingoing current and plain arrows the outgoing current. Figure 10 shows an open oral mouth with ingoing ciliary tracts indicated by broken arrows and the outgoing ones by complete arrows. On either side of the outgoing tract in the mouth there are two tracts of cilia which beat inward and continue into the ingoing ciliary tract on the roof of the arm canal. There is always a copious secretion of mucus, and material is carried along the tracts in mucous strings. In the axial canal (ax.) cilia cover the whole surface and beat in an outward direction, but there is no tract of cilia beating inward and no ciliary reversal was observed (fig. 9). Eggs were seen carried by cilia down the axial canal and then toward the center of the oral disc.

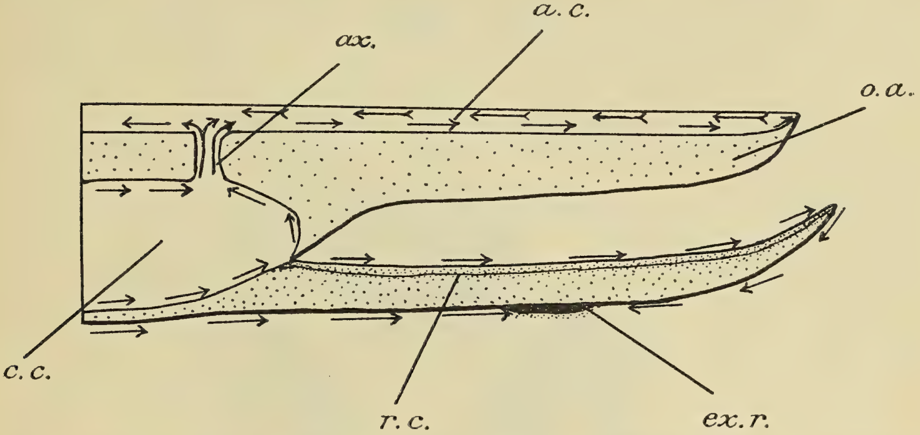

FIG. 9—Diagrammatic section passing between gonads to show ciliary currents. Feathered arrows indicate the ingoing ciliary current and plain arrows the outgoing current in the oral arm. ax., axial canal; ex.r., ring on ex-umbrella; o.a., oral arm. Other lettering as before.

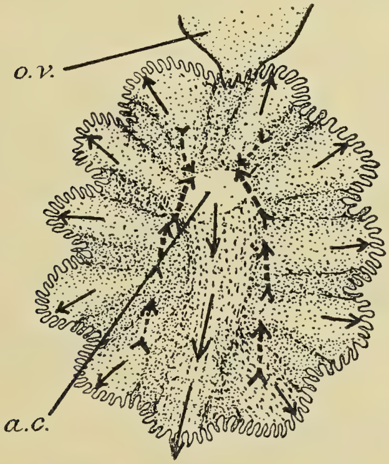

FIG. 10—Expanded oral mouth to show the ciliary currents. Ingoing ciliary currents are indicated by broken arrows, the outgoing tract by complete arrows. Lettering as before.

Within the cœlenteron the grooves between the plaited membranes possess cilia which beat outward to the openings of the axial canals, and on the ex-umbrella side the cilia also beat in an outward direction, as shown in figures 9 and 11. Cilia cover the surface of the plaited membranes. Those on the inner side of the gastric filaments carry particles toward the outgoing currents in the grooves. On the outer side the cilia beat in the opposite direction for some distance, then the direction changes and the particles are carried across to the lateral edges of the plaited membrane and along the edges toward the openings of the axial canal as indicated by the arrows in figure 11. The gastric filaments and radial canals are ciliated, and on the former the beat of the cilia is in the direction of their attachment to the plaited membrane. In the radial canals the ciliary currents could not be worked out in detail, but evidence was obtained that some of them can carry particles away from the cœlenteric end to the anastomosing branches, while in others the cilia beat toward the cœlenteron.

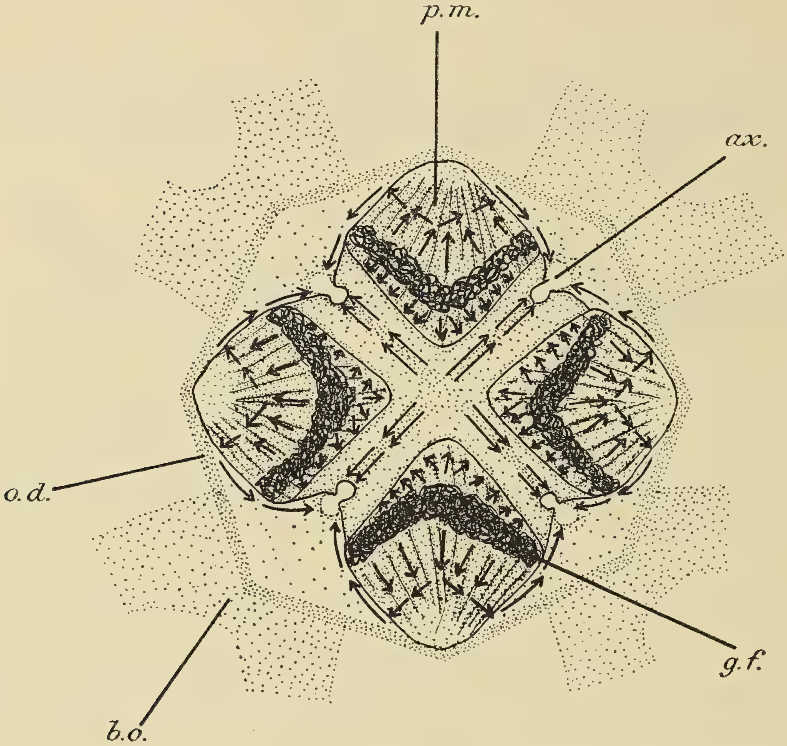

Fig. 11—Ciliary currents of lenteron, ex-umbrella being removed to expose plaited membranes. b.o., base of oral arms; p.m., plaited membrane. Other lettering as before.

EXTERNAL CURRENTS

Cilia cover practically the whole of the external surface. On the arms the cilia beat outward and in the direction of the tentacles on the oral mouths (fig. 12, A, B). On the inside of the oral mouth, below the tentacles and above the ingoing ciliary tracts, the cilia beat outward as shown in figures 10 and 12, B. Rejected matter is carried by these cilia to the tips of the tentacles where it drops off. Carmine particles placed on the sub-umbrella are carried outward to the edge of the animal and over the edge to the ex-umbrella surface. On the ex-umbrella surface the cilia within the ring (ex. r.) of slightly elevated and wrinkled ectoderm beat outward, while the cilia on the edge beat inward toward the ring (figs. 9, 13). In this way waste matter and débris collect in the form of a ring, which is thrown off when the animal starts to swim. The cilia which line the sub-genital chambers beat outward.

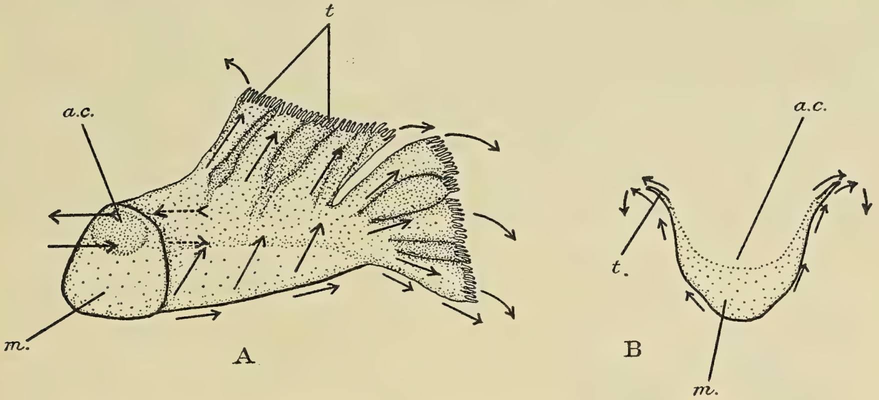

FIG. 12—External ciliary currents of the oral arm and mouth. A, side view. B, section through oral mouth. Lettering as before.

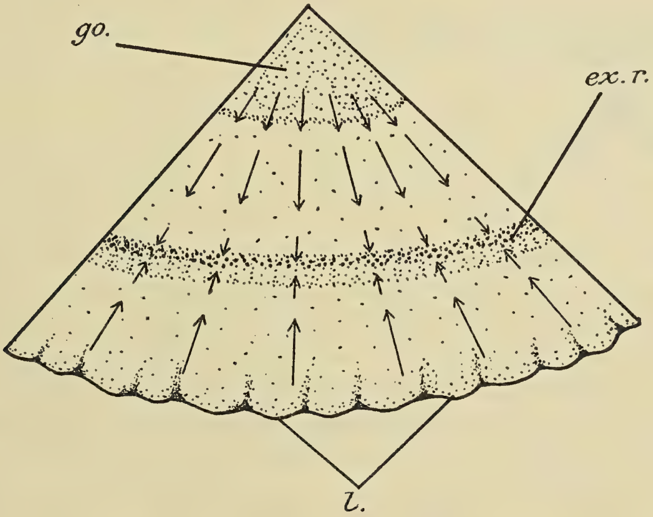

FIG. 13—The ciliary currents of the ex-umbrella. go., gonad showing through ex-umbrella tissue. Other lettering as before.

TRANSPORT OF FOOD

When placed in an oral mouth, food is caught in the ingoing ciliary current and carried along the roof of the arm canal into the axial canal leading to the cœlenteron. The method of transference of food into the cœlenteron was at first puzzling, since there is no ingoing ciliary current in the axial canal. It was seen later, however, that the gastric filaments are responsible for this. These are arranged in little groups to form a comparatively broad band across the plaited membrane. The plaited membrane is capable of muscular movements, although no trace of muscle fibers was seen in sections, and the presence of food causes them to contract toward the center of the disc, thus bringing the gastric filaments in contact with the opening of the axial canals (figs. 14, A, B). During feeding, the dumb-bell-shaped opening of the axial canal (ax.) into the cœlenteron contracts and the central parts meet to form an inner and an outer opening. The gastric filaments enter the inner opening and wrap themselves round the food particles as they enter the axial canal from the canals of the arms. The mucous strings of food are thus caught in the ciliary currents of the gastric filaments so that even when the filaments are withdrawn from the opening they may still continue to pull in the food strings.

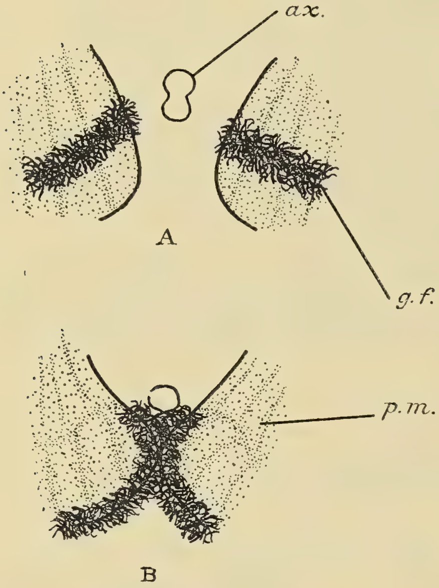

Fig. 14—Gastric filaments and plaited membrane. A, position of filaments before feeding. B, arrangement of gastric filaments during feeding. Lettering as before.

REJECTION OF PARTICLES

Rejected particles and waste matter are carried across the plaited membrane to the grooves leading to the axial canals and so to the canals of the arms. When the filaments occupy the inner opening, the particles are carried by way of the outer opening, but when the filaments are withdrawn the particles enter the axial canal by both openings. A suspension of carmine introduced at the cœlenteric openings is carried in a continuous stream of mucus along the whole length of the arm canal and ejected by the oral mouths at the extreme tips of the arms. Normally, however, all the oral mouths are capable of passing out waste matter. If food particles fall upon the sub-umbrella they are carried by ciliary currents to the margin. Usually the arms flatten out and press against the sub-umbrella, with the tips almost in contact with the margin, and when the food particles reach the edge they are captured by the tentacles on the arms. Otherwise the particles are carried over the edge to the ring on the ex-umbrella side of the medusa and later cast off with the waste matter collected there.

EFFECT OF FEEDING AND SEDIMENTATION ON THE RATE OF PULSATION

A marked difference in the rate of pulsation was observed in *C. frondosa* before and after feeding. No variation in the rate followed the act of feeding, but some 30 minutes later the animals became inactive and the pulsations very irregular and infrequent. After about 80 minutes the animals became active again, and apart from a few irregularities the pulsations were again normal.

A mechanical stimulus such as the deposition of sediment also brings about a change in the beat. The normal rate of pulsation of two animals was found to be 44 and 60 beats per minute. Sand was then poured on the animals and it was found that the rate of pulsation increased to 66 and 84 beats per minute, respectively, about 1½ times the normal rate. The animals also endeavored to raise themselves from the bottom to swim. The swimming action takes place only when a large amount of silt falls on the animals. Small quantities are removed by ciliary action.

DIGESTIVE ENZYMES

Although the physiology of digestion in the Hydrozoa and Anthozoa has attracted the attention of many workers, comparatively little is known of these processes in the Scyphozoa. Bodansky and Rose (1922) working on *Stomolophus meleagris* found a number of enzymes in tissue extracts of the gastric filaments. Of these, two are proteases—a "pepsin" with an optimum at pH 3, a "trypsin" at pH 7.3, an amylase, a weak lipase, a maltase, and a very weak invertase. Bodansky and Rose confined their investigations entirely to tissue extracts, so the presence of extracellular protease in the cœlenteric fluid of the Scyphozoa remained in doubt. Chuin (1929, a), however, has shown that in the scyphistoma of *Chrysaora* proteins are broken down in the cœlenteron by an acid, viscous secretion. Mayor (1906) noticed that the cœlenteric fluid of *C. xamachana* was slightly alkaline, but thought that was due to the presence of the zooxanthellæ. Extracellular digestion of protein takes place universally in the Hydrozoa and Anthozoa and it will be shown below that it occurs also in *C. frondosa* and that the digestive processes are essentially the same as those of other cœlenterates.

The methods adopted follow closely those of Yonge (1930, b) in his work on the digestive enzymes of the Madreporaria. The cœlenteric fluid was obtained by carefully pipetting through the ex-umbrella side of the cœlenteron. Tissue extracts were made of the gastric filaments and the plaited membrane at the base. It was hoped that it might be possible to obtain pure extracts, but the presence of a few zooxanthellæ was unavoidable. Extracts were made in fresh, filtered sea-water and kept until required in a refrigerator. Before use they were filtered until a clear fluid was obtained. Toluol was used to prevent bacterial action, both in the extracts and during subsequent experiments. The enzymes are very resistant to high temperatures and required prolonged heating before they were destroyed. In the experiments on the optimum pH range of proteases, the enzymes in the controls were not entirely destroyed, and as limitations of time did not permit further experiments the results in these cases have been put forward in the absence of controls.

THE pH OF THE FLUID IN THE CŒLENTERON

Experiments made on the pH of the fluid in the cœlenteron are summarised in table 1. The pH was determined by placing drops of the fluid from each of a number of animals on a white plate, mixing suitable indicators with them and comparing the resultant colors with indicator standards.

Table 1—*pH of cœlenteric fluid.*

No.	Time after feeding.	pH
1	0 mins.	7.5
2	10 mins.	7.4
3	20 mins.	7.3
4	30 mins.	7.3
5	40 mins.	7.1
6	1 hr.	7.4
7	1 hr. 20 mins.	7.6
8	1 hr. 30 mins.	7.9
9	1 hr. 40 mins.	7.8
10	2 hrs.	7.9
11	3 hrs.	8.0
12	4 hrs.	8.1

pH of sea-water=8.2

Allowing for some individual variation, a flushing-out process evidently takes place in the cœlenteron. From table 1 it can be seen that within the first hour of digestion the pH of the cœlenteric fluid drops to 7.1, while after 4 hours it becomes almost as alkaline as sea-water. The pH of the cœlenteric fluid of a starved specimen was compared with that of a specimen 4 hours after feeding, the former was 7.8, the latter 8.0, indicating that alkaline sea-water had entered the cœlenteron after digestion had been completed. The normal pH in the cœlenteron of a starved specimen is about 7.8, and in this it agrees with the condition in Madreporarian corals (Yonge, 1930, b). Yonge also found that the pH in the cœlenteron of these corals dropped to about 7.05 after feeding, which closely corresponds with the lowest pH recorded for *Cassiopea.* In *Hydra,* Beutler (1927) found that the pH of the cœlenteric fluid is normally weakly alkaline but rises to 8.2 after feeding.

THE PROTEOCLASTIC ENZYMES

In the Cœlenteric Fluid

Eleven specimens were fed for 15 minutes with mollusc meat, and 30 minutes after feeding the cœlenteric fluid was pipetted out and made up to 80 c.c. with fresh, filtered sea-water. The pH of the fluid was 7.2. Fibrin was dissolved after 8 days and gave a strong Biuret reaction. A digest of peptone was also prepared and both were tested with Millon's reagent for tyrosine after 14 days with negative results.

To determine the pH range for the action of extracellular protease, a number of digests were made with varying amounts of acid, alkali and distilled water and 0.05 gm. fibrin added to each, 1 c.c. being removed for pH estimation. The experiment was carried out over a period of 16 days and the stages of dissolution of fibrin indicated numerically, *e.g.* 0 = no dissolution and 10 = complete dissolution. The results are contained in table 2.

Table 2—*pH range for the action of protease in the cœlenteron.*

No.	pH	Days												
		4	5	6	7	8	9	10	11	12	13	14	15	16
A	4.7	0	0	0	0	0	0	0	0	0	0	0	0	0
B	5.7	0	0	0	0	0	0	½	1	2	3	4	5	6
C	6.7	0	0	½	1	2	3	4	5	5½	6	6½	7	8
D	7.0	½	1	2	3½	5	6½	7½	8½	9½	10	10	10	10
E	7.3	½	1	2	3	4	5	6½	7½	8½	9½	10	10	10
F	7.8	0	½	1	1½	2	3	4	5	6	7½	8½	9½	10
G	8.0	0	½	½	1	2	3	4	5	6	7	8	9	10
H	8.4	½	½	1	2	3	4	5	6	7	8	9	10	10
I	9.0	½	1	2	3	4	5	6	6½	7	8½	9½	10	10

The results are shown graphically in figure 15, and it can be seen that there are two optima, one about pH 7.0 and the other about pH 9.0. The first corresponds closely to the pH of the cœlenteron within the first hour of digestion. Almost identical results have been obtained by Yonge (1930, b) for *Fungia.* Vonk and Heyn (1929) have shown that there is a second optimum at pH 11.3 for the action of trypsin on fibrin, due to the effect of hydrogen ion concentration, but in any case, as Yonge points out, since the second optimum pH never actually occurs in the cœlenteron, it can be disregarded. Yonge also tested the digests at the two optima for tyrosine with negative results.

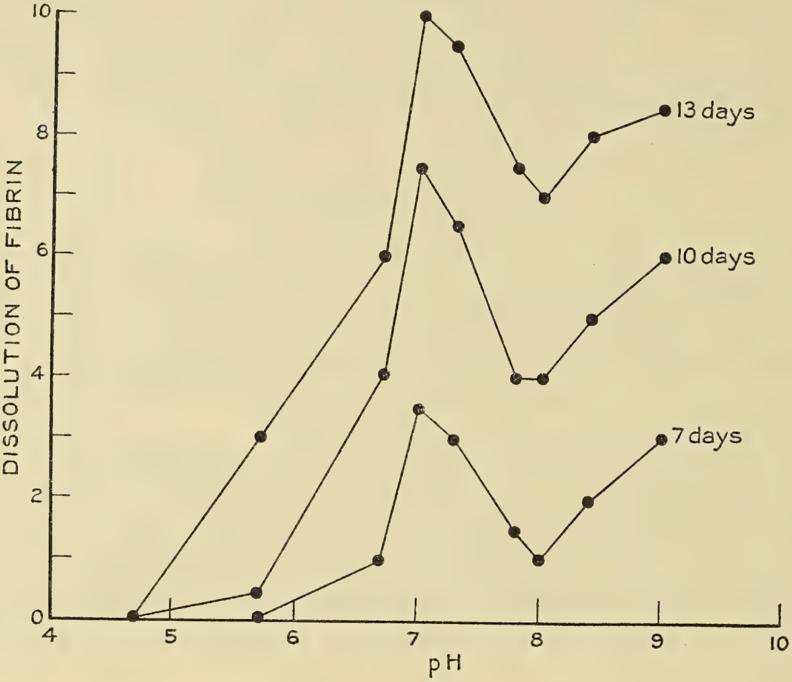

FIG. 15—Graph showing dissolution of fibrin at various pH by protease from cœlenteric fluid. See table 2.

IN TISSUE EXTRACTS

Extracts of tissue from 8 specimens were made up to 120 c.c., the pH of the fluid being 6.7. When tested for the production of amino-acids with peptone and fibrin, the presence of tryosine was revealed after 6 days.

The pH range for the action of intracellular protease on fibrin was determined in a similar manner to that of the extra-cellular protease. An extract from 15 specimens was made up to 150 c.c. and 5 c.c. of the fluid were used in each case with distilled water and acid or alkali to make 11 c.c.; 1 c.c. was removed for pH determination and 0.05 gm. fibrin added to each. The results are contained in table 3.

The graph for the dissolution of fibrin (fig. 16) shows 2 optima, one at pH 7.1 and the other about pH 9.0. The first optimum agrees with the optimum pH for the extra-cellular protease and is due, no doubt, to its presence in the extract. Bodansky and Rose (1922) found two proteoclastic enzymes in *Stomolophus*, one closely resembling pepsin with an optimum about pH 3.0 and the other a trypsin with an optimum about pH 7.3. Unfortunately, the range of pH values recorded above excludes any possibility

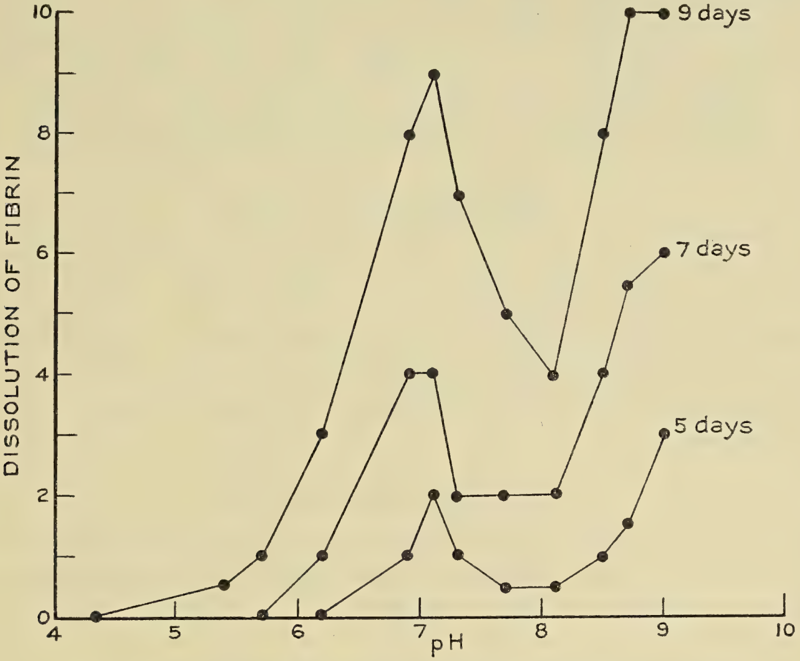

FIG. 16—Graph showing action on fibrin at various pH of proteases from extracts of the gastric filaments. See table 3.

TABLE 3—*pH range of proteases acting on fibrin.*

No.	pH	Days															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A	3.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	4.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C	5.4	0	0	0	0	0	0	0	0	½	½	1½	2	2½	3	3½	4
D	5.7	0	0	0	0	0	0	0	½	1	2	3	4	5	5½	6	6½
E	6.2	0	0	0	0	0	½	1	1½	3	5	6	7	8	8½	9	10
F	6.9	0	0	½	½	1	1½	4	5½	8	9	9½	10	10	10	10	10
G	7.1	0	½	½	1	2	3	4	6	9	10	10	10	10	10	10	10
H	7.3	0	0	0	½	1	1½	2	3	7	8	9	10	10	10	10	10
I	7.7	0	0	0	0	½	1	2	3	5	7	8	9	10	10	10	10
K	8.1	0	0	0	0	½	1	2	3	4	6	7½	9	9½	10	10	10
L	8.5	0	0	0	½	1	2	4	6	8	9	10	10	10	10	10	10
M	8.7	0	½	½	1	1½	3	5½	7	10	10	10	10	10	10	10	10
N	9.0	½	½	1	2	3	4	6	7	10	10	10	10	10	10	10	10

of demonstrating the presence of a "pepsin" in *C. frondosa.* Yonge (1930, b) on *Lobophyllia* covered a wide range of values from pH 2.2 to 10 and failed to show the presence of any enzyme resembling pepsin. The results obtained with *Lobophyllia* are in agreement with those obtained with

C. frondosa in that both show in the tissue extracts the presence of an extracellular protease with its optimum about neutrality and an intracellular protease active between pH 8 to 10. It is probable that the protease with an optimum about pH 7.3 found in *Stomolophus* is due to the presence of the extracellular protease, for the results obtained by Bodansky and Rose show a slight increase in the velocity of digestion about pH 8 which might have become more evident had these investigators extended their range.

THE LIPOCLASTIC ENZYMES

No lipoclastic enzymes were found in the cœlenteric fluid. An extract of tissue from 15 specimens was made up to 150 c.c. with filtered sea-water and 20 drops of an emulsion of olive oil added to 20 c.c. of the extract. A control was also set up and both titrated after 16 days with 0.1 N NaOH, 3 c.c. being required for the former and 1 c.c. for the latter. A difference of 2 c.c. was obtained, thus demonstrating the presence of lipoclastic enzymes in the extract.

THE SUCROCLASTIC ENZYMES

Tests for the presence of sucroclastic enzymes in the fluid of the cœlenteron gave negative results. Extracts were tested for various sucroclastic enzymes, the results being given in table 4.

TABLE 4—*Presence of sucroclastic enzymes in the extract.*

Extract	Substrate	Time	Result
5 c.c.	5 c.c. 1 per cent starch	6 days	Reduction with Fehling's soln. (slight).
	Control	"	No reduction.
5 c.c.	5 c.c. sat. soln. glycogen.	6 days	Reduction (strong).
	Control	"	No reduction.
5 c.c.	5 c.c. 5 per cent sucrose	16 days	No reduction.
	Control	"	No reduction.
5 c.c.	0.1 gm. cellulose	16 days	No reduction.
	Control	"	No reduction.
5 c.c.	5 c.c. 2 per cent lactose	16 days	No reduction with Barfoed's soln.
	Control	"	No reduction.

Of the five carbohydrates tested, only starch and glycogen gave positive reactions, thus showing the presence of an amylase and a glycogenase. The reaction for amylase was very weak, while the glycogenase was very strong. These sucroclastic enzymes were the only ones found by Yonge in extracts of *Lobophyllia* and he has been able to show that the amylase originated from the zooxanthellæ and not from the coral. *Dendrophyllia nigrescens*, a coral which does not contain algæ, was found to possess no amylase. In

Cassiopea also the very weak amylase reaction is probably due to zooxanthellæ. Verification of the presence of an amylase in the zooxanthellæ of *Cassiopea* was not difficult because the algæ are very abundant in the oral arms and it was possible to obtain an extract without fear of contamination from the gastric filaments. The extract was made from two specimens and made up to 75 c.c., the pH being 6.7. Tests with Fehling's solution after 7 days showed a strong positive reaction for the experiment with starch and a slight positive reaction with glycogen. It appears, then, that in *Cassiopea* glycogenase is the only sucroclastic enzyme, of those tested, the zooxanthellæ being responsible for any trace of amylase found in extracts.

ASSIMILATION AND EXCRETION

It has been shown that extracellular digestion of protease takes place in the cœlenteron of *Cassiopea* in which food matter is broken down into fine particles. It now remains to be seen how and where these particles are ingested, digestion completed and finally where excretion takes place. The most important work on this subject in the Cœlenterata is that of Yonge (1931, a) on the Madreporaria where he has demonstrated that absorption and excretion take place in the same area, namely the inner region of the mesenterial filaments. Mouchet (1930), who worked on excretion alone, has obtained similar results for the Actiniaria. Pratt (1905) has found that ingestion of carmine particles in the Alcyonaria is accomplished by the endodermal cells of the mesenterial filaments, body wall, and endodermal canals, and she suggested that the cells of the mesoglœa form an amœboid plexus of strands for distribution of nutriment throughout the colony and for the conveyance of waste products to the cœlentera or to the canals. Chuin (1929, b, c) has shown that endodermal cells lining the cœlenteron are concerned with digestion in the scyphistoma of *Chrysaora*. The endodermal cells are vacuolated at their free ends and when food is introduced they pour out a secretion. He has also worked on the cytological changes that occur in these cells during digestion. Apart from Chuin's work, little is known of these processes in the Scyphozoa.

A number of *Cassiopea* were fed with fish blood. The corpuscles were quickly taken in, their presence in the cœlenteron being denoted by a pink tinge seen through the ex-umbrella. The medusæ were then fixed in Bouin and Flemming and sections cut. An examination of sections revealed a number of blood corpuscles about the gastric filaments in specimens which had been fed for 15, 30, and 45 minutes. Figure 17 shows the blood corpuscles (b.c.) surrounded by mucus (muc.) and many of them in the act of ingestion by cells of the gastric filaments and by endodermal cells at the base of the filaments. There are numerous empty nematocysts and gland cells and some of the gland cells could be seen in the process of discharging their secretion. Yonge (1931, a) has found that the granular gland cells in the Madreporaria secrete the extracellular enzymes. Digestion by the extracellular protease evidently takes place rapidly, for the cytoplasm of corpuscles in the canals of the arms stain a bright pink with eosin, while in the cœlenteron the eosin staining is very faint and the cell walls are very irregular in shape. A few corpuscles were also seen to be ingested by cells of the plaited membrane over the gonad.

Partially digested corpuscles could be seen inside the gastric filaments as darkly staining masses within vacuoles and some contained in wandering cells were seen to be passing into the mesoglœa (fig. 17). In a specimen fed for 1 hour and fixed 2 hours later, a few wandering cells with the remains of blood corpuscles were found in the mesoglœa of the gastric filaments and below the plaited membrane at the base of the filaments. Flemming fixed sections were examined to ascertain the course of digestion of the blood corpuscles, but shrinkage of the mesoglœa rendered this attempt unsuccessful. Fat, however, is abundant generally in the tissues. There was no sign of glycogen in material fixed in Carnoy and treated with iodine.

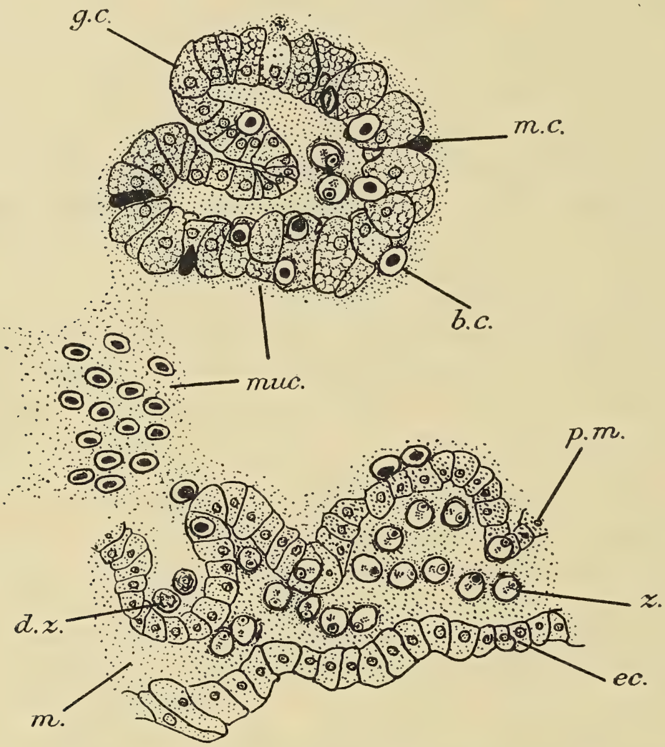

Fig. 17—Section through a gastric filament and plaited membrane at base after being fed for 45 minutes with blood corpuscles. x 675. b.c., blood corpuscle; d.z., degenerating zooxanthellæ; g.c., granular cell; muc., mucus.

A fine suspension of carmine was injected into the mesoglœa of the umbrella of medusæ, special care being taken that none should come in contact with the gastric filaments or plaited membrane. They were then washed in fresh sea-water, fixed at different times in Bouin and later sectioned. After 4 hours carmine particles were seen in the endoderm at the base of the gastric filaments (fig. 18, B). After 24 hours carmine particles could be seen excreted from the gastric filaments and the plaited membrane. In the mesoglœa a few wandering cells were seen containing carmine particles (fig. 18, A).

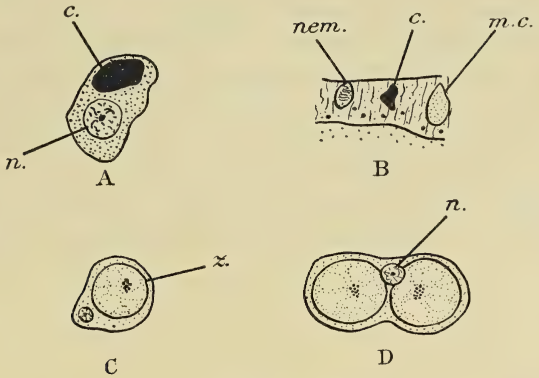

FIG. 18—A, wandering cell from mesoglœa carrying carmine particle 24 hours after injection with carmine. B, carmine particle seen in the endoderm of the plaited membrane 16 hours after injection with carmine. C, and D, wandering cells from the mesoglœa containing zooxanthellæ. x 1250. c., carmine. Other lettering as before.

Absorption and excretion take place, therefore, in the gastric filaments, the endoderm at the base of the filaments and, to a slight extent, in the rest of the endoderm forming the plaited membrane. Food material is carried throughout the tissues and waste products removed by wandering cells but there is no evidence of the amœboid plexus described by Pratt, in the Alcyonaria.

THE ZOOXANTHELLÆ

STRUCTURE AND DISTRIBUTION

The occurrence of zooxanthellæ in many species of the Scyphozoa has been noted by many workers whose records have been reviewed by Buchner (1930). Among the Semæostomeæ only *Linuche draco* (Thiel, 1927) and *L. unguiculata* var. *aquila* (Mayer, 1910) are known to possess zooxanthellæ, in saccules of the umbrella between the gonads, but they occur in many species of the Rhizostomeæ. The algæ were thought by Hamman (1881) to be gland cells, while Haeckel (1879) supposed they were spermatozoa, but their true nature was later established by Geddes (1882) and Brandt (1883). In *Cotylorhiza* (Claus, 1884) they occur in the endodermal cells and in the anastomosing canals of the gastro-vascular system, but in the majority of the Rhizostomeæ they are situated in the mesoglœa where they usually occur in groups. The distribution seems to vary in different species, for in many cases the groups of zooxanthellæ are described as being situated just below the ectoderm and nowhere else in the mesoglœa as, for example, in *Mastigias papua* (Uchida, 1926, a). In *C. xamachana* (Bigelow, 1900) they are situated throughout the mesoglœa.

Bigelow has shown that the zooxanthellæ possess cellulose walls and starch, but little is known of their structure. In *C. frondosa* the structure has been investigated and the algæ found to be identical with those described by Yonge and Nicholls (1931, a) in the Madreporaria. They are spherical, yellowish-brown cells bounded by a stout cellulose wall and measuring from 6 to 12 μ in diameter, the majority being about 9 μ. The nucleus is large and granular, and close to it is the assimilation product and pyrenoid. Sometimes there are two assimilation products and pyrenoids in a single zooxanthellæ. The cytoplasm is very vacuolated and contains numerous oil drops which stain black in material fixed with Flemming (fig. 19). The chloroplasts could not be seen in fixed preparations as the yellow-brown pigment dissolves out very rapidly leaving them colorless. The zooxanthellæ are to be found in abundance throughout the whole of the mesoglœa but especially in the neighborhood of the oral mouths. A few were seen also in ectodermal and endodermal tissue. As in other Rhizostomeæ they usually occur in groups in the mesoglœa. Examination of macerated tissue and sections showed that they are carried about by wandering cells (fig. 18, C, D) which may contain, one, two or even more.

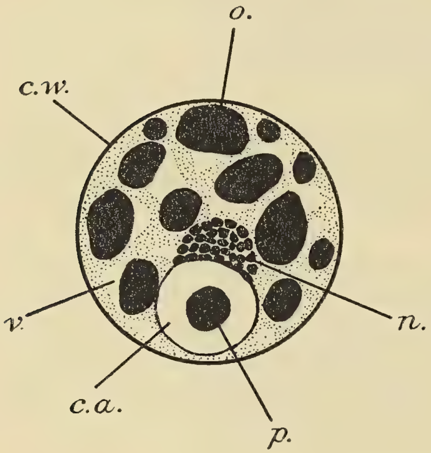

FIG. 19—A zooxanthella. x 5000. c.a., clear area; c.w., cell wall; o., oil drop; p. pyrenoid. Other lettering as before.

PHYSIOLOGY

The utilization by the zooxanthellæ of carbon dioxide and nutrient salts is well known and extensive experiments have been carried out in the Madreporaria by Yonge and Nicholls (1931, a, b) and Yonge, Yonge, and Nicholls (1932) on this subject. They have shown that in the presence of light, the amount of oxygen in the sea-water surrounding the corals increased, while the phosphates and nitrates diminished, the pH remaining constant.

At night the amount of oxygen diminished, while with the consequent increase of carbon dioxide in the water there was a drop in pH. When the metabolism of the corals was lowered by starving, heating, or deprivation of oxygen, the amount of nutrient salts and carbon dioxide produced by the animals was diminished and this was followed by the expulsion of numerous zooxanthellæ. When kept in darkness, for a time all the zooxanthellæ were expelled and the animals remained in a healthy condition for many weeks in the absence of zooxanthellæ, and it was concluded that the association between corals and algæ is essential to the plants but not to the individual coral colonies. The only physiological work on this matter in the Scyphozoa has been done by Mayor (1906). Mayor kept *C. xamachana* alive for one month in absolute darkness, and although the plant-cells degenerated, the medusæ did not suffer, and he therefore concluded that the animal's vitality is not dependent on the zooxanthellæ. The general effects of starvation have also been described by Mayor (1914) and by Cary (1917).

The following experiments were made with *C. frondosa.*

Starved in Light

Four vessels were used for this experiment, each holding 2 litres of fresh, filtered sea-water and one medusa was placed in each. The water was changed each day, but at the end of 8 days all had died. A plunger tank, holding about 12 litres of sea-water, was then set up and 4 specimens placed

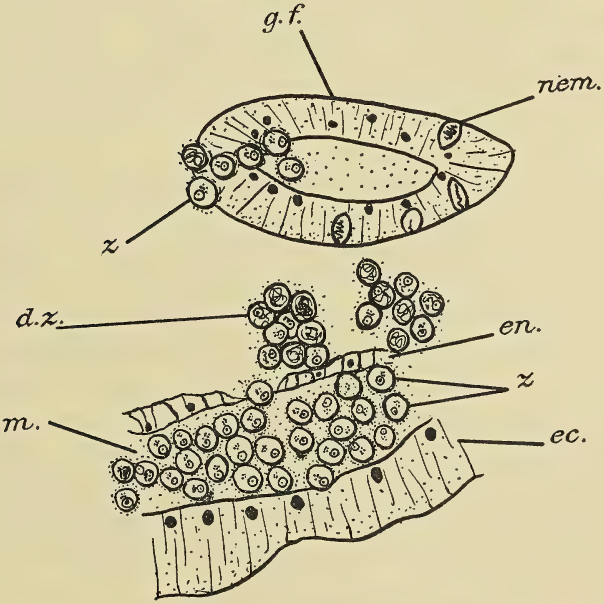

FIG. 20—Section through a gastric filament and plaited membrane at the base after starving in light for 15 days. x 600. Lettering as before.

in it and the water changed every day. After 10 days they were quite healthy, but had become a darker brown and numerous algæ were extruded. At the end of 15 days they were still living but smaller and a very dark brown in color, and in one animal the gonads and plaited membranes had disintegrated. The experiment was then stopped and the animals fixed. Subsequent sectioning showed that the zooxanthellæ were collected in masses beneath the ectoderm and endoderm all over the body and many had already died. This would account for the dark-brown color of the animal. No algæ were seen in the deeper parts of the mesoglœa and the whole appearance was similar to that of *Mastigias papua* (Uchida, 1926, b). Zooxanthellæ were especially numerous at the base of the gastric filaments and many were being ejected in that region and in the gastric filaments (fig. 20). A few zooxanthellæ were also found to be ejected by endodermal cells of the oral arms and by ectodermal cells, but this is, perhaps, an unusual phenomenon brought about by experimental conditions.

Starved in Darkness

A medusa was placed in each of 4 vessels with 2 litres of fresh filtered sea-water and kept in a cool part of the aquarium in a light-tight box. The water was changed each day, but on the third day three of the animals died. The water was then changed twice a day and the remaining animal lived for 7 days, when it was fixed. At the beginning of the experiment the specimen was 5 cm. in diameter, but at the end of the experiment it measured only 2.5 cm. and was in a very unhealthy condition. In another experiment three medusæ were kept in darkness and the water changed twice a day but by the eighth day all the medusæ had died and were beginning to disintegrate.

Mayor (1914) starved *C. xamachana* and found it resulted in no appreciable chemical change although the cells become reduced in size, many of them degenerating and disappearing while the cell boundaries tend to become indistinct. The mesoglœa also becomes vacuolated and muscular tonus is lost so that the bell rim bends upward and inward. Mayor found that the oral mouths and vesicles are the first organs to disappear, and points out that after starving for 3 weeks reorganization of tissue may be impossible, owing to inability to capture zooplankton. In addition Mayor found that the medusæ become dark brown in color after 10 days starvation. The effects of starvation in *C. frondosa* are in agreement with those described by Mayor. Starvation also brings about absorption of the gonad along with other tissues, but the gastric filaments are not reduced in any way.

Effect on Phosphorus Excretion

Two medusæ were used for this experiment and kept in separate vessels each containing 2½ litres of fresh filtered sea-water. The phosphorus was estimated as phosphate and the figures for phosphorus obtained by calculation. The initial amount of phosphorus in the sea-water was 4.37 mg. per cubic meter. In one vessel the phosphorus content went to zero after 24 hours, while in the other it increased to 25.35 mg. per cubic meter. There was no change in the phosphorus content of the control after 24 hours. In the vessel showing an increase in phosphorus there was a considerable quantity of mucus, and gonad had been cast off by the animal which, no doubt, accounts for the increase. No mucus was seen in the vessel showing a decrease in phosphorus content of the water. A number of similar experiments were carried out, but in all cases mucus was given off a short time after the experiment was started and the phosphorus results were higher than the initial phosphorus in the sea-water.

Nitrate estimations were attempted also but these were not successful.

DISCUSSION

The tendency toward a sedentary habit is very marked in *C. frondosa,* as in other Rhizostomeæ, and has brought about a number of interesting structural modifications. Compared with the Semæostomeæ it is a heavy, rather solid animal, and for that reason is a very weak swimmer and able only to maintain the swimming movements for a few minutes, sinking, eventually, to the bottom. In *C. xamachana* the raised ring and concave surface of the ex-umbrella act as a sucker helping the medusa to adhere to the substratum (Perkins, 1908), and a similar arrangement exists in *C. frondosa* although it is not so obvious. Owing to the sedentary habit, the area devoted to feeding is greatly developed, resulting in numerous feeding funnels or oral mouths. The medusa is carnivorous and possesses a highly developed feeding mechanism, the most remarkable being the oral vesicles. These act as powerful organs of offense and defense and frequently great irritation has been set up in the soft skin of the hands when handling the animals. Although nematocysts are of universal occurrence in the Cœlenterata, no other case, as far as is known, has been described in which they are localized in masses and shot out at the prey.

Apart from the inner area of the ring of the ex-umbrella, the whole of the external and internal surface of *C. frondosa* is covered with cilia, and as mucous secretion is always associated with cilia, it is not surprising to find mucous cells present in large numbers in the ectoderm and endoderm. Duerden (1906) has stressed the importance of mucus in corals in protecting the surface from foreign objects and keeping it clean, and also in entanglement and ingestion of prey. This applies also to *Cassiopea.* Food is carried in mucous strings by cilia along the roofs of the oral arms to the axial canals where they are collected by the gastric filaments. There are no ingoing ciliary tracts in the axial canals, and only by the aid of the filaments can the food gain entry to the cœlenteron so that, besides being the chief organs of digestion, they also play an important part in the feeding mechanism. Widmark (1911, 1913) has worked out the internal ciliation of *Aurelia aurita* and he has found that there are outgoing currents in the interradial and perradial canals. Indications of a similar condition were found in *C. frondosa.* On the floor of the cœlenteron of *Aurelia* there are ingoing tracts from the mouth opening to the gastric filaments. No such tracts exist in *Cassiopea.* In both animals particles are carried to the lateral edges which lead to the openings to the oral arms. The main difference in the internal ciliation is due to the fact that in *Cassiopea* the central mouth has disappeared so that particles enter and leave the cœlenteron by a common opening, while in *Aurelia* they do so by separate openings. Gemmill (1920) has worked on the ciliation of the ephyra of *Aurelia* and found also that prey is caught by the nematocysts on the lappets which bend toward the mouth and wipe their captures on to the lips of the mouth. Orton (1922) has described a similar mode of food capture in the adult. Small organisms from the plankton are collected on the surface of the ex-umbrella and partly by ciliary action and partly by contraction of the bell they are transferred to the fringe of the umbrella. Food is also collected on the sub-umbrella and carried to the margin where it is collected at the middle of each segment to form 8 blobs of food which are licked off by the oral arms and passed into the gastric pouch. This mode of feeding differs, then, from *Cassiopea* in which the capture of food is accomplished by the oral vesicles and oral mouths. Moreover the cilia on the margin of the umbrella of *Cassiopea* beat in the opposite direction to those of *Aurelia,* waste matter being carried from the sub-umbrella over the lappets to the ring on the ex-umbrella, where it is later discarded. Such an arrangement is necessary in *Cassiopea* where the deposit of foreign bodies is more likely to constitute a danger to the well-being of the animal.

Extracellular digestion takes place in the cœlenteron where food matter is broken down to polypeptides by an extracellular protease with an optimum about pH 7.0 secreted by the granular cells of the gastric filaments. Assimilation of food particles takes place on the gastric filaments and plaited membrane at the base of the filaments and later they are carried by wandering cells through the mesoglœa. Tissue extracts contained in intracellular protease capable of breaking down protein and polypeptides to amino-acids and with an optimum about pH 9.0. Fat, glycogen, and starch can be digested by enzymes from the extracts, but the amylase has been shown to originate from the zooxanthellæ. The digestive processes in *Cassiopea* are therefore similar to those in Madreporaria (Yonge, 1930, b).

It is generally held that in the Rhizostomeæ "extra-intestinal" digestion takes place within the oral mouths. This idea is based on work by Hamann (1881), which is also given prominence in a review of extra-intestinal digestion by Lengerken (1924). Hamann states that larger prey are grasped by the oral arms which form a funnel-shaped space and there they are broken down by extracellular enzymes to fine particles to be taken into the cœlenteron later by the openings of the sucking canals. He also states that small objects are digested in the oral mouths and that indigestible substances do not enter the cœlenteron. In his opinion the stomach juice is passed to the oral mouths and so enables digestion to take place as described above. The cœlenteric fluid certainly passes along the arm canals to the mouths in *Cassiopea,* but only by the outgoing ciliary tracts and therefore will not come in contact with food. It has been impossible to confirm Hamann's observations. Food is always taken in to the cœlenteron before digestion begins and this has been shown by feeding with blood corpuscles. When sectioned later, the corpsucles in the oral arms, on their way to the cœlenteron, were definitely not attacked by any enzyme and showed a marked contrast in appearance when compared with corpuscles actually inside the cœlenteron. The oral arms are capable of great distension and can carry large copepods from the plankton quite easily. Indigestible matter is also taken into the cœlenteron provided the medusa is first of all given a food stimulus.

In the cœlenteron the pH of the fluid varies during and after digestion. About 40 minutes after feeding the pH drops to 7.1 which is about the optimum pH for the extracellular protease. About 4 hours after feeding, the cœlenteric fluid becomes almost as alkaline as sea-water, later dropping to about pH 7.8. After digestion, therefore, a flushing-out process takes place in the cœlenteron. The rate of pulsation also varies during and after digestion. About 30 minutes after feeding, the pulsations practically cease, but after 80 minutes the animals become active again. The cause of pulsation in *C. xamachana* and the effect of chemicals on the rate of pulsation have been investigated by Mayor (1908, 1917) and the effect of temperature on the rate of pulsation of *Rhizostoma pulmo* by Uexkull (1901). Mayor by adding water of pH 6 was able to slow down the rate of pulsation, while with the addition of water of pH 8 the rate was maintained or increased. Weese and Townsend (1921) have studied the reactions of *Æquoria æquoria* in different gradients of pH and found that the jellyfish showed a preference for sea-water of pH 7.9 and that it became inactive when the pH reached 8.8 or 6.8. Now the radial canals in *Cassiopea* communicate directly with the marginal sense-organs and are concerned only in maintaining a circulation of water throughout the umbrella. It is suggested, therefore, that the pH of the cœlenteric fluid may control the activity of the medusa in natural life, especially as a correlation is indicated between the experiments on *Cassiopea* and the work of Mayor and of Weese and Townsend. It is noteworthy that *Æquoria* showed a preference for sea-water of pH 7.9 which is about the normal pH of the cœlenteron when digestion is not taking place. The lower pH during digestion might well be the cause of the slowing of the rate of pulsation in *Cassiopea* during this period.

The gastric filaments and plaited membranes are the main sites of excretion, but the ejection of zooxanthellæ in the case of starved specimens has been seen in ectodermal and endodermal cells in other parts of the body. In the Madreporaria, absorption and excretion take place *only* at the mesenterial filaments, so that although a similar condition has been found in *Cassiopea*, the arrangement is less specialized in the latter.

The association with zooxanthellæ is especially interesting, for in *Cassiopea* they are mainly confined to the mesoglœa, although a few are to be found in the cells of the ectoderm and endoderm. In the Cœlenterata, with the exception of the Scyphozoa, when zooxanthellæ occur they do so only in the cells of the endoderm and never in the ectoderm. As, however, the algæ are carried about by wandering cells which are to be found in large numbers in the mesoglœa of the Scyphozoa it is not surprising to find the zooxanthellæ also in the mesoglœa. The zooxanthellæ usually occur in isolated groups with often large numbers in a single wandering cell, but the mechanical difficulty in their transportation through the mesoglœa may account for this.

A considerable amount of literature has been published on the nature of the association between invertebrates and unicellular algæ and the whole matter has been discussed by Yonge (1934). In the Madreporaria (Yonge, 1931, b), the zooxanthellæ are excreted when the metabolism of the corals is lowered and they are not utilized as food. Their importance lies in their ability to rid the corals of their waste products very rapidly, although it has been shown that individual corals can live quite well without them. Practically nothing is known of the nature of the association in the Rhizostomeæ, although Browne (1905) suggests that they are parasites. The zooxanthellæ have been shown to be similar in structure to those contained in the Madreporaria and also the fact that in one experiment the phosphorus in the sea-water was reduced within 24 hours to zero indicates that the waste products of the medusa are utilized by the unicellular plants for protein synthesis. The digestive enzymes of *Cassiopea* are specialized for acting on animal matter so that the zooxanthellæ probably can not be digested by their host. There is no evidence that food in solution is not passed from the zooxanthellæ to the wandering cells but, on the other hand, when starved in light and in darkness there is always a vigorous expulsion of zooxanthellæ which seems to point to the fact that food is not derived from the zooxanthellæ. The medusæ could only be kept alive when starved for a very short period, but Mayor (1906, 1914) working with *C. xamachana*, a much hardier species, starved specimens for a month in darkness. At the end of that time they were still healthy and he concluded, therefore, that the animals are not dependent on the zooxanthellæ.

It is not known how the jellyfish acquire the algæ. From the egg to the planula stage no zooxanthellæ are present and none have been observed in the early scyphistomæ of *Mastigias papua* (Uchida, 1926, a) and of *C. frondosa*. They occur in the scyphistoma of *C. xamachana*, and Bigelow (1900) in his description of the process of budding in the scyphistoma states that the algæ are passed into the buds and the resultant planulæ therefore contain zooxanthellæ. In the ephyra stage of *M. papua* and *C. xamachana*, zooxanthellæ are abundant. Presumably the zooxanthellæ are incorporated by late scyphistomæ from prey containing them, such as coral planulæ, especially as Marshall (1932) has found that these planulæ may contain many hundreds of these algæ.

In conclusion, then, the nature of the association between *C. frondosa* and zooxanthellæ appears to be similar to that found in Madreporaria, namely the algæ subserve the function of excretory organs but do not provide nutriment to their host. Also the association is not so specialized as in the Madreporaria, because the zooxanthellæ are not present in the planulæ and are only acquired when the young reach an actively feeding stage.

SUMMARY

1. *C. frondosa* is a specialized carnivore.
2. The oral arms possess numerous small mouths and vesicles. These oral vesicles are concerned with the capture of food and have small bags of nematocysts and mucous cells which they shoot out at passing crustaceans.
3. Food is carried to the axial canals by cilia on the roof of the oral arm and there it is caught by the gastric filaments. The cilia lining the axial canals do not beat toward the cœlenteron.
4. Waste matter is carried across the plaited membranes to the lateral edges and so to the axial canals. From the axial canals it is carried along the floor of the oral arm to the exterior.
5. Rejected particles are carried to the ring on the ex-umbrella, where they are later thrown off.
6. The pH of the cœlenteric fluid varies during and after feeding and shows that a flushing-out process takes place in the cœlenteron. Forty minutes after feeding, the pH of the fluid is about 7.1, and 4 hours after feeding it increases to about 8.1. In the case of a starved medusa the pH of the fluid is about 7.8.
7. Digestion of food is begun in the cœlenteron by an extracellular protease secreted by the granular cells of the gastric filaments and having an optimum pH of about 7.0. The extracellular protease breaks down proteins to the polypeptides but not to amino-acids.
8. In extracts of the gastric filaments and plaited membranes a number of enzymes were found: a protease with an optimum about pH 9.0 which can break down proteins and polypeptides to amino-acids, a lipase, a glycogenase and an amylase. The amylase has been shown to originate from the zooxanthellæ.
9. Assimilation and excretion take place in the gastric filaments and plaited membranes.
10. Nutriment is carried to different parts of the body, and excretory products to the gastric filaments and plaited membranes, by wandering cells which pass through the mesoglœa.
11. Zooxanthellæ occur mainly in the mesoglœa and are contained in wandering cells.

12. Experiments have been made on the effect of starvation in light and in darkness on the medusæ. In light, specimens were kept alive for 15 days, and in darkness for 7 days.

13. Numerous algæ were ejected by way of the gastric filaments and plaited membranes at the base of the filaments and the medusæ became brown in color. They also shrunk considerably in size.

14. The effect of the zooxanthellæ on phosphorus excretion has been studied, the amount of phosphorus in the sea-water surrounding one specimen being reduced to zero within 24 hours.

15. Finally, feeding, digestion and symbiosis in *C. frondosa* have been discussed. It has been suggested that the variation in pH in the cœlenteron effects the activity of the jellyfish. The association with zooxanthellæ is probably similar in nature to that which occurs in the Madreporaria.

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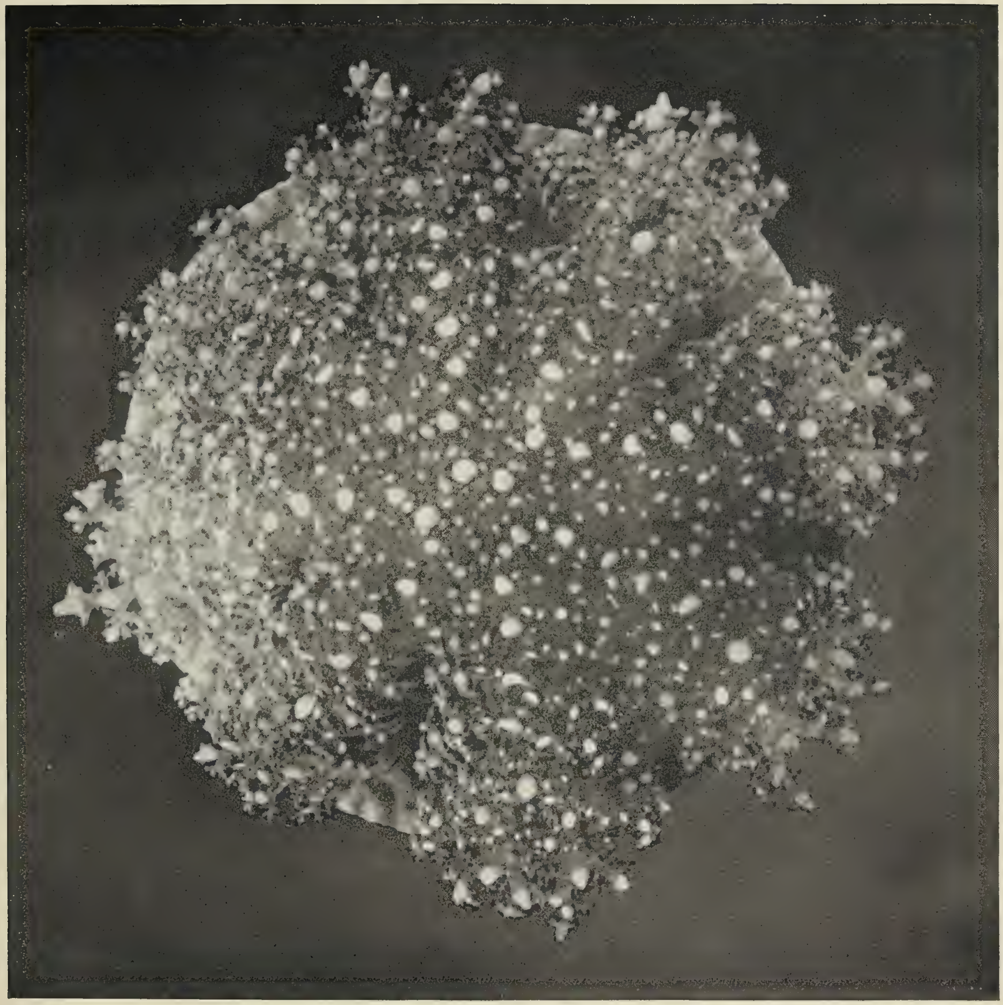

Oral view of *C. frondosa.*

PAPERS FROM TORTUGAS LABORATORY, VOL. XXXI

III

THE WATER EXCHANGES OF LIVING CELLS

IV: Further Studies on the Water Relations of the Eggs of the Sea-urchin, Echinometra lucunter

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University of California

With five graphs

[Issued July 24, 1936.]

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THE WATER EXCHANGES OF LIVING CELLS

IV: Further Studies on the Water Relations of the Eggs of the Sea-urchin, Echinometra lucunter

The application of a photographic method to the determination of the non-solvent volume of the eggs of the sea-urchin, *Echinometra lucunter,* and also the effect on the non-solvent volume determinations of the length of time of immersion of eggs in diluted sea-water solutions, was demonstrated in another publication (Leitch, 1936). The present paper considers (1) the utilization of this photographic method for the study of the swelling and shrinking of the eggs of the same sea-urchin, (2) the effect on the water relations of these cells of the time between that of spawning and that of introducing the eggs into the experimental solutions, and (3) an analysis of equations which have been developed and applied by several investigators to explain the kinetics of water exchanges of living cells.

Grateful acknowledgment is made to Professor S. C. Brooks of the University of California for his critical reading of the manuscript, and to Mr. Harold Leitch for his assistance with the mathematical phase of the paper.

MATERIALS

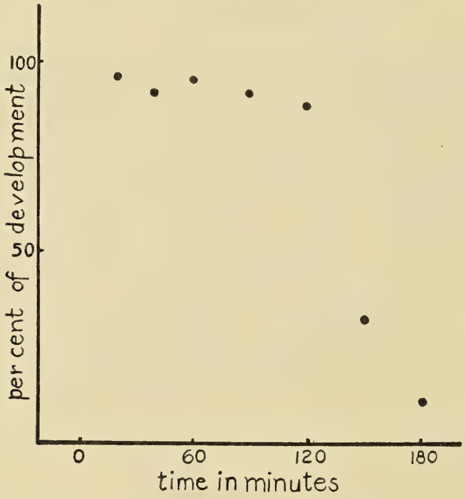
The eggs of the sea-urchin, *Echinometra lucunter,* were obtained by inverting each animal in a large finger bowl of sea-water. Ten to fifteen minutes later spawning occurred in the majority of the specimens brought into the laboratory. Upon the inception of spawning, the males were transferred to a dry finger bowl, since sperm when diluted with sea-water quickly lost their viability, a reaction noted by Hayes (1933). Those animals not spawning under the above conditions were opened and the eggs obtained by breaking the ovaries in a dish of sea-water. By repeated washing with filtered sea-water and centrifugation, the majority of the tissue débris was removed. Such eggs, although appearing identical with those obtained by natural spawning, were found to react differently during experimental work to be described below.

EXPERIMENTS

The experimental work will be presented under five headings, namely: (1) fertilization experiments, (2) non-solvent volume experiments, (3) swelling experiments, (4) shrinking experiments, and (5) reactions of eggs not spawned spontaneously. In each series, duplicate determinations were made on each sample of eggs, one involving measurements of the egg diameters using the filar ocular micrometer and the other employing the photographic method (Leitch, 1936). The capacity of the eggs for development was ascertained after each experiment by returning the eggs to normal sea-water and adding 2 or 3 drops of freshly spawned sperm.

FERTILIZATION EXPERIMENTS

To check the effect on fertilization and development of allowing the eggs to stand in sea-water before introduction into the experimental solutions, the eggs from one female were placed in finger bowls containing 100 cubic centimeters of sea-water and allowed to stand at room temperature (27° to 31°C.). At intervals, from 20 minutes after the time of spawning, a small sample of the egg suspension was transferred to another dish of sea-water and 2 or 3 drops of freshly spawned concentrated sperm suspension was introduced and thoroughly mixed. For one experiment, the percentage of developing eggs, determined by counts of the swimming larvæ, is plotted in Graph 1 against the time elapsing from spawning. In all other similar experiments, the same decrease, as indicated by the decided break in Graph 1, was observed in the percentage of eggs developing after standing 2 hours in sea-water. If the sperm used in these experiments had also been allowed to stand for approximately the same length of time as the eggs, the break in the curve would have come between 40 and 60 minutes.



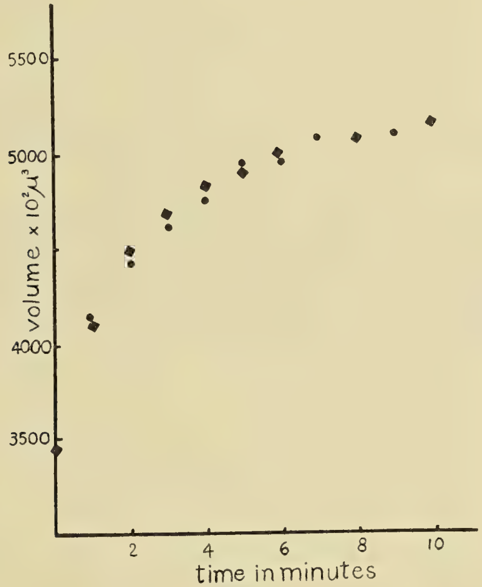
Graph 1—Relationship between the per cent of development of the eggs of *Echinometra lucunter*, as determined by actual counts of number of larvæ, and time elapsing from spawning.

NON-SOLVENT VOLUME EXPERIMENTS

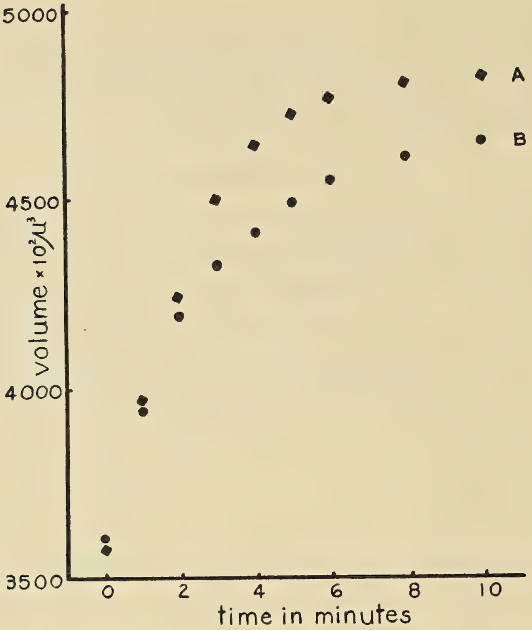
Eggs were transferred from normal sea-water, in which they had been standing for varying periods of time, to diluted sea-water solutions and allowed to reach equilibrium. Measurements of eggs were made after an exposure to these solutions of from 70 to 80 minutes, a time at which equilibrium is established and prior to the period of vacuolization (Leitch, 1936). These data are summarized in table 1 (p. 61).

SWELLING EXPERIMENTS

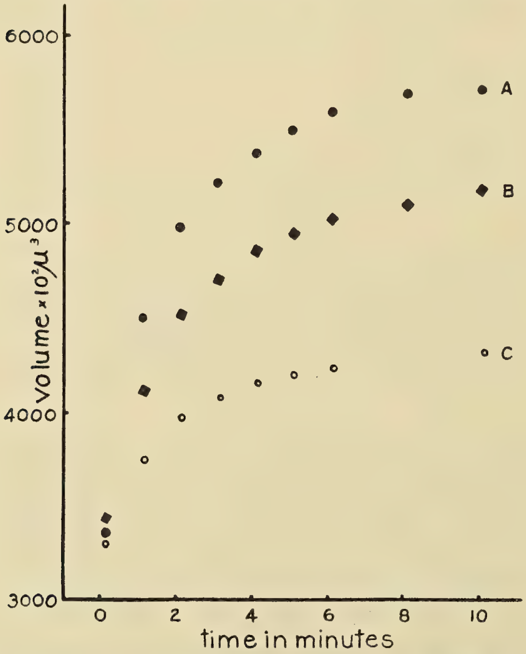
In the swelling experiments, a small sample of a suspension of eggs, which after being spawned had stood for a definite time at room temperature, was introduced into 50 cubic centimeters of a dilute sea-water solution which in the several experiments ranged in concentration from 70 per cent to 50 per cent that of normal sea-water. The temperature was maintained approximately constant at 28° Centigrade during all swelling and shrinking experiments by placing the dish containing the experimental material in a larger dish of water to which ice-cooled water was added from time to time. Graph 2 represents the volume-time relationships for two experiments, one involving measurements by the filar ocular micrometer and the other by photography. Examples of the swelling curves of eggs when placed in 60 per cent sea-water solutions at increasing periods of time after spawning are given in Graph 3, while in Graph 4 are given the swelling curves of eggs in 70, 60 and 50 per cent sea-water.



Graph 2—Volume-time curves of the eggs of ***Echinometra lucunter*** swelling in 60 per cent sea-water as determined by filar micrometer measurements (indicated in graph by ●) and by measurements of photographs (indicated in graph by ◆).



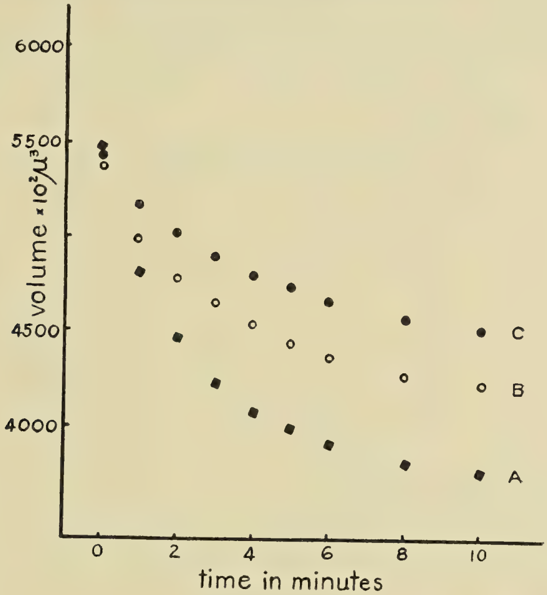
GRAPH 3—Effect of time elapsing from spawning on the swelling-time curves of the eggs of *Echinometra lucunter* in 60 per cent sea-water. Curve A represents values of eggs which have stood for 2½ hours after spawning. while Curve B is for eggs which have stood for 4¼ hours.



GRAPH 4—Swelling-time curves of the eggs of *Echinometra lucunter* when allowed to swell in 50 per cent (Curve A), 60 per cent (Curve B), and 70 per cent sea-water solutions (Curve C).

SHRINKING EXPERIMENTS

In the study of the exosmosis of water from the egg cell a small volume of egg suspension was placed in a dilute sea-water solution. After 75 minutes, a time sufficient for attainment of equilibrium (Leitch, 1936), the eggs were allowed to settle of their own weight, first in a tall cylinder and finally in a 15 cubic centimeter centrifuge tube. The supernatant dilute solution was pipetted off, the remaining small volume of egg suspension returned to normal sea-water, and the eggs allowed to shrink. The resulting changes in volume are plotted against time in Graph 5. It will be seen again that allowing the eggs to stand before subjection to the experimental solutions produces a notable effect on the volume-time curve.



GRAPH 5—Volume-time curves of the eggs of *Echinometra lucunter* shrinking in normal sea-water after having stood in 60 per cent sea-water at room temperature for 30 minutes (Curve A), 2½ hours (Curve B), and 4¼ hours (Curve C).

REACTIONS OF EGGS NOT SPAWNED SPONTANEOUSLY

When eggs which had been removed experimentally from the ovaries were placed in dilute sea-water solutions, they became crenated during the first two or three minutes of exposure. Later in the swelling process there was a slight tendency for the eggs to assume a spherical shape, but not to a degree sufficient to permit accurate measurements. The general irregularity of outline eliminated all possibility of using these eggs for the determinations of non-solvent volume, swelling or shrinking characteristics. This reaction appears to be related to some physico-chemical change occurring at the time the eggs are liberated by the animal into the sea-water. Explanation of this phenomenon must await further studies on this phase of the problem.

DISCUSSION

FERTILIZATION EXPERIMENTS

In earlier work, the formation of fertilization membranes had been assumed to be an indication that cells used in experiments were uninjured, but the appearance of fertilization membranes was found to be an unreliable criterion of normality. In several experiments this reaction occurred in nearly 100 per cent of the eggs, but no further development ensued, a phenomenon similar to that observed in Pacific Coast forms (Leitch, unpublished data). Consequently, the formation of normal swimming larvæ has been considered the ultimate criterion of an uninjured condition of the experimentally treated eggs.

In checking the normality of eggs following their exposure to experimental solutions, it was found that as the time elapsing from spawning increased, there was a decrease in the percentage of eggs which developed normally (Graph 1). This occurred irrespective of the concentration of the diluted sea-water used, and appeared to be a reaction dependent on changes in the eggs rather than an effect of experimental procedure. The experiments outlined above on the relationship between the number of free-swimming larvæ and the time elapsed from spawning indicate that the eggs remain in a normal condition for approximately 2 hours, subsequent to which there is a sharp decrease in the number which will produce larvæ. This is shown in Graph 1 where the percentage of developing eggs, as determined by counts of the swimming larvæ in one experiment, is plotted against the time elapsing from spawning. The break in the curve (Graph 1) occurs at approximately the same time as that of a series of protoplasmic changes which can be observed under the microscope. The egg protoplasm becomes more transparent with a volume increase due, apparently, to a break-down in the cell constituents [1] and an increased osmotic pressure of the egg which results in an intake of water. The normal spherical shape of the eggs then becomes distorted and a reaction, similar to that of pale cytolysis, begins and finally results in the disintegration of the entire cell. With a horizontal microscope one sees, in eggs which have stood for 2 or more hours in sea-water, an increased flattening of the cells which, at the beginning of the period of cytolysis, amounts to approximately 10 per cent. This flattening suggests that changes are also occurring in the cell surface which might also involve changes in its permeability to water. That these changes can in part be retarded for several hours has been shown by keeping the eggs at a temperature of from 2° to 4° Centigrade.

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[1] The conclusion that the increased volume of the eggs at this time is associated with a break-down of cell constituents which produces an increased osmotic pressure inside the egg will be further substantiated by the results of chemical analyses to be published in a succeeding paper.

NON-SOLVENT VOLUME EXPERIMENTS

The non-solvent volume of the eggs of *Echinometra lucunter* was calculated by the method described by Leitch (1936). The effect of the elapsed time from spawning on the non-solvent volume of the eggs is clearly indicated from the results of experiments given in table 1. The non-solvent volume of the eggs remains relatively constant at an average value of 34 per cent of the initial volume for almost 2 hours, while on longer standing before use the value is increased to 53 per cent. In experiment No. 12, however, the eggs following spawning had been placed in the ice box (2° to 4° C.) and samples removed at the time given in table 1. Then, upon determining the non-solvent volume, the values were found to agree closely with those derived from measurements made on eggs directly following spawning. However, tests of the capacity of these eggs to produce normal larvæ showed that, although the lowered temperature had apparently retarded the cytolytic changes previously described for eggs standing for a much shorter period of time at a higher temperature, such cells were also unable to develop beyond the stage of membrane formation. Although involving equilibrium conditions rather than active swelling, table 1 offers further proof of the conclusion (Leitch, 1936) that photography can be employed in the study of the water exchanges of living cells.

Table 1—*Effect of time elapsing from spawning on values of non-solvent volume of eggs of Echinometra lucunter.*

Exp. No.	Type of exp.	Non-solvent volume as per cent of initial volume	Time from spawning
1	Photographic	32.2	69 mins.
2	Photographic	33.8	101 mins.
3	Ocular	53.5	225 mins.
6	Ocular	32.3	57 mins.
7	Photographic	52.9	175 mins.
10	Photographic	37.9	43 mins.
12	Ocular	36.5 34.0 36.8	2 hrs. 3.5 hrs. 5 hrs.

EQUATIONS ON THE KINETICS OF WATER EXCHANGES

All equations so far developed for the quantitative calculation of the permeability of a cell to water are based on the assumption that the rate of volume change with time is dependent upon (1) the area permeable to water, which is considered to be equal to the total area of the cell surface at any time; (2) the thickness of the plasma membrane which is unknown and is, therefore, combined in the constant of the equation; and (3) the difference in activities across the plasma membrane which is taken as proportional to the difference between the osmotic pressure of the egg protoplasm and that of the external medium. The general equation for the permeability may therefore be written as follows:

$$dV/dt = K_1S\ (P - P_e) \tag{1}$$

in which dV/dt is the rate of change in volume at time, t; S, the area of the cell surface at time, t; P, the osmotic pressure of the egg protoplasm at time, t; P_e, the osmotic pressure of the external medium and, therefore, that of the egg at equilibrium; and K_1 the permeability constant (K/h) which includes the factor, h, for thickness of the plasma membrane and therefore the values of this constant apply only to the eggs under consideration. If it could be shown that the thickness of the plasma membrane were the same in all eggs, then the constant would have the same significance for all forms as the relationship between K_1 and K would be identical in all cases.

Further development of equation (1) by previous workers assumes diverse forms depending upon whether S is considered constant or variable and also upon the point in the integration at which volumes are substituted for osmotic pressures. Lillie (1916) after considering S as constant and making the necessary substitutions after integration gives the following equation:

$$K_2t = \ln \frac{V_e - V_o}{V_e - V_t} \tag{2}$$

in which V_e, V_o, V_t are, respectively, volumes at equilibrium, at the beginning of swelling and at time, t, during swelling, and K_2 is an arbitrary constant.[1] (In all other equations where these symbols occur, they have the same significance as in equation (2). In subsequent equations in this paper this involves a few changes in the subscripts from those given in the original references.) In developing equation (2) Lillie substitutes $1/V$ for P. However, these two factors are not identical since P actually equals P_oV_o/V.

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[1] The actual derivation of equation (2) was made by Lillie, using the equation for the course of an unimolecular reaction as a basis rather than equation (1) (See Lillie, 1916). The latter may be used if S is considered to be constant and if the rate of volume change is proportional to the total amount of swelling which will occur divided by the distance from the equilibrium condition.

Northrop derives two equations from equation (1), the first (1927a) assuming that S is constant while the second (1927b) takes S as variable. Since the second equation resembles that derived by Lucké, Hartline and McCutcheon (1931), any further description of it here is omitted. Northrop's equation, however, although similar to equation (2), since both consider S as constant, involves the substitution of volumes for pressure prior to rather than after integration to give:

$$K_3t = \frac{hV_e}{SP_o}\left(V_o - V_t + 2.3\, V_e \log \frac{V_e - V_o}{V_e - V_t}\right) \tag{3}$$

in which K_3 is the number of cubic micra of water passing through 1 square micron of surface per minute under an osmotic pressure difference of 1 atmosphere providing volume, surface and osmotic pressure are expressed in terms of cubic micra, square micra and atmospheres, respectively. This equation was developed by Northrop primarily to explain the passage of water through a collodion membrane, and is here introduced solely for comparison with an equation to be developed later.

Lucké, Hartline and McCutcheon (1931) consider that S is continually varying during the experiment and express it as a function of the volume so that equation (1) becomes:

$$K_4 = \frac{V_e \qquad dV/dt\ V_t^{1/3}}{(36\pi)^{1/3}\ P_oV_o \quad (V_e - V_t)} \tag{4}$$

in which K_4 is the permeability constant and has the same dimensions as K_3 of equation (3). Instead of considering the osmotic pressure as proportional to the observed total volume, they consider it to be proportional to the osmotically active volume and derive a second relationship:

$$K_5 = \frac{(V_e - b)\ dV/dt\ (V_t - b)}{(36\pi)^{1/3}\ P_o(V_o - b)\ V_t^{2/3}\ (V_e - V_t)} \tag{5}$$

in which b equals the volume of osmotically inactive material. For the complete equations following integration the reader is referred to the original papers (Lucké, Hartline and McCutcheon, 1931 or Lucké and McCutcheon, 1932). If equations (4) and (5) are used, it is possible to determine the value of K_4 and K_5 for each minute of the swelling and shrinking curve, but when the completely integrated equations are used the constant must be determined graphically from the slope of the line resulting from plotting the complex volume expression against the time. This latter method gives only an average value for the constant for each experiment and does not permit the determination of any changes in the permeability constant as swelling progresses. This fact will be considered later following the discussion of the remainder of the equations.

The writer considers that equation (1), after substituting the corresponding volumes for the pressure factors and taking S as constant, results in the following:

$$K_6 = \frac{V_e\,(dV/dt)\,V_t}{SP_o\,V_o\,(V_e - V_t)} \tag{6}$$

which on integration becomes:

$$K_6t = \frac{V_e}{S\,P_oV_o}\left(V_o - V_t + V_e \ln \frac{V_e - V_o}{V_e - V_t}\right) \tag{6a}$$

There is a great similarity between equations (3) and (6a), but the latter differs in containing in the constant term the factor V_o, the presence of which is absolutely essential since it is involved in the substitution of volumes for pressures prior to integration. Although S is considered as a constant, equation (6) permits determination of the effect of surface area on the permeability constant, K_6.

In expressing the pressure factors of equation (1) in terms of the corresponding volumes, the values used for the latter in equation (6) were the observed total volumes. However, since a certain portion of the volume is osmotically inactive, it was thought advisable to express equation (1) in terms of osmotically active rather than total volumes, to give:

$$K_7 = \frac{(V_e - b)\,(dV/dt)\,(V_t - b)}{S\,P_o\,(V_o - b)\,(V_e - V_t)} \tag{7}$$

which on integration becomes:

$$K_7t = \frac{V_e - b}{S\,P_o\,(V_o - b)}\left(V_o - V_t + (V_e - b)\ln \frac{V_e - V_o}{V_e - V_t}\right) \tag{7a}$$

Jacobs and Stewart (1932) have developed three other equations for the study of cell permeability which will not be considered in this paper, as they involve the special case in which a penetrating solute is also entering the cell at the same time as water.

APPLICATION OF THE EQUATIONS TO SWELLING AND SHRINKING OF ECHINOMETRA EGGS

The permeability constants of the eggs of *Echinometra lucunter* in 70, 60 and 50 per cent sea-water, as calculated from equations (2), (4), (5), (6) and (7), are summarized in table 2. The corresponding swelling curves have already been given in Graph 4 (p. 58). In the case of equations (2), (4a), (5a), (6a), and (7a), the values of the corresponding constants can be determined graphically by plotting the right side of the equation against the time. The latter method gives the average value of each constant for any one experiment, and these results are given in table 3 under the column headed "Graphical." For purposes of comparison, the values of the permeability constants for the third minute[1] and of the average of the values in table 2 are also given in table 3.

TABLE 2—*Permeability constants, as calculated from equations (2), (4), (5), (6) and (7), for eggs of Echinometra lucunter for first six minutes of swelling in 70, 60 and 50 per cent sea-water solutions.*

Time in minutes	K_2			K_4			K_5			K_6			K_7		
	70 p. ct.	60 p. ct.	50 p. ct.	70 p. ct.	60 p. ct.	50 p. ct.	70 p. ct.	60 p. ct.	50 p. ct.	70 p. ct.	60 p. ct.	50 p. ct.	70 p. ct.	60 p. ct.	50 p. ct.
1	0.578	0.472	0.614	0.356	0.476	0.508	0.291	0.415	0.466	0.388	0.526	0.615	0.317	0.466	0.565
2	.531	.462	.535	.308	.379	.376	.257	.343	.353	.349	.453	.486	.291	.411	.458
3	.475	.402	.458	.304	.287	.406	.256	.261	.387	.350	.351	.542	.295	.321	.516
4	.426	.389	.418	.285	.280	.402	.240	.257	.385	.331	.350	.547	.279	.322	.524
5	.439	.361	.391	.327	.278	.437	.278	.256	.421	.385	.352	.604	.326	.325	.581
6	.414	.350	.391	.331	.303	.482	.282	.281	.466	.392	.388	.674	.332	.359	.652

TABLE 3—*Permeability constants of eggs of Echinometra lucunter in 70, 60 and 50 per cent sea-water solutions as determined graphically from integrated forms of equations (2), (4), (5), (6) and (7). For comparative purposes, constants for third minute and average of values for all six minutes from table 2 are included.*

Permeability constant	70 per cent			60 per cent			50 per cent		
	Graphical	At 3d. minute	Average of 6 minutes	Graphical	At 3d. minute	Average of 6 minutes	Graphical	At 3d. minute	Average of 6 minutes
K_2	0.378	0.475	0.477	0.315	0.402	0.406	0.318	0.458	0.468
K_4	.314	.304	.319	.320	.287	.334	.382	.406	.435
K_5	.193	.256	.267	.185	.261	.302	.285	.387	.413
K_6	.370	.350	.366	.292	.351	.403	.545	.542	.578
K_7	.308	.295	.307	.270	.321	.367	.505	.516	.549

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[1] This is the procedure proposed by McCutcheon and Lucké (1928) who maintain that the values for the preceding minutes are somewhat inaccurate, due to the difficulties encountered in measuring diameters while the eggs are very rapidly increasing in volume.

The permeability constants (table 2) show a decided decrease between the first and third minutes followed by somewhat more constant values for the last four minutes. In 50 per cent sea-water, however, the values for the sixth minute are higher than at the previous three minutes and are either approximately equal to or slightly greater than those for the first minute. There appears to be about the same degree of variation in the permeability constants for all the five equations considered in table 2. In no case is the permeability constant a true constant, for it varies not only in different dilutions of sea-water but even at different minutes in any one dilution. Goldforb (1935) finds a progressive increase in the permeability constants for *Arbacia* eggs. Lucké, Hartline and McCutcheon (1931) have shown that for the eggs of *Arbacia punctulata*, the permeability constants are more nearly identical for experiments in solutions of varying osmotic pressures if the volume factors in equation (4) are taken as the osmotically active volumes. (This equals the observed volume minus the non-solvent or osmotically inactive volume.) In the values of K_5 and K_7, the equations in which the non-solvent volume correction has been introduced, no better agreement is obtained between these constants and the corresponding K_4 and K_5 with variations in the osmotic pressure of the experimental solutions. From a mathematical standpoint, the introduction of a constant into equations (4) or (6) should change all values by the same amount and should not bring about any better correspondence between the experiments involving solutions of different osmotic pressures. However, if the non-solvent volumes vary with the external osmotic pressure, then it would be possible for the permeability constants to be approximately identical in all experimental solutions, irrespective of the dilution. Previous workers (Leitch, 1934, and McCutcheon, Lucké and Hartline, 1931) have shown that the non-solvent volume is independent of the concentration of the external solution. However, Ponder (1934, Chap. V.) has stated that in the case of the mammalian red-blood cells, the non-solvent volume does actually vary with the external osmotic pressures but appears to be constant. This apparent constancy is due to a loss of solutes by the cells which varies with the dilution of the external medium. McCutcheon, Lucké and Hartline (1931) have maintained that the eggs of *Arbacia punctulata* after exposure to hypotonic solutions on being returned to normal sea-water resume their initial volume, and conclude, therefore, that these eggs do not lose solutes during the course of the experimental procedures. However, it would seem quite possible to consider that these results are due to a loss of solutes during exposure to the hypotonic solutions followed by an equal gain of solutes on returning the eggs to normal sea-water. Further work on this phase of the water relations of the eggs of *Echinometra lucunter* is being undertaken.

In constants K_6 and K_7 which were calculated on the assumption that the area permeable to water remains constant and equal to the initial area of the cell surface, the values are of the same order of magnitude as those of K_4 and K_5, in which the permeable area is assumed to increase with the volume. However, although in all experiments there is a tendency for the former constants to be slightly higher than the latter, this increase is so small that it is of doubtful significance. This clearly indicates that any assumption concerning the changes in the area permeable to water has little or no significance until a time when the exact relationship between this factor and the total area of the cell surface is known.

The values of the permeability constants as determined from the integrated equations and summarized in table 3 show just as much variation with varying hypotonicity of the external solutions and in the same solutions at different time intervals as is found in table 2. However, a slight decrease exists in the limits of the constants in table 3 from those in table 2, that is from 0.250 to 0.650 in the latter to 0.180 to 0.580 in the former (these values are in terms of the number of cubic micra of water passing through one square micron of cell surface per minute under an osmotic pressure gradient of one atmosphere). Due to the observed variations and overlapping of values of the constants in other experiments, such a difference seems hardly significant.

From a study of tables 2 and 3, it is immediately apparent that no one equation is any better suited than any other to explain the swelling of the eggs. Consequently the values obtained can only be considered as semi-quantitative.

TABLE 4—*Permeability constants of eggs of Echinometra lucunter when transferred to normal sea-water and allowed to shrink after having reached equilibrium in a 60 per cent sea-water solution.*

Time in minutes	K_2	K_4	K_5	K_6	K_7
1	0.457	0.209	0.146	0.192	0.134
2	.414	.164	.112	.143	.0981
3	.396	.139	.0936	.117	.0789
4	.378	.133	.0882	.109	.0726
5	.347	.125	.0828	.102	.0673
6	.361	.129	.0843	.103	.0675

Table 4 summarizes the permeability constants for eggs which, after having reached equilibrium in 60 per cent sea-water, were returned to normal sea-water and allowed to shrink. The values of the constants have been calculated, using the same equations by means of which the results in table 2 were obtained. In the case of equation (2), the constants for swelling and shrinking are practically identical and show a comparable variation with time. Constants derived from the use of equations (4), (6) and (7) give much lower values for experiments involving the shrinkage of eggs than for those involving swelling, which would indicate that some factor operating during exosmosis and not during endosmosis has not been considered in the derivation of these equations. In the case of the eggs of *Arbacia punctulata*, Lucké, Hartline and McCutcheon (1931) found that the introduction of the correction factor, *b* (the non-solvent volume), produced fairly close agreement between the results of swelling and shrinking experiments. But for the eggs of *Echinometra lucunter*, the use of such a correction (in equations (5) and (7)) does not harmonize the values obtained from data on swelling and shrinking experiments. This leads to the important conclusion, a point sometimes overlooked in biological work, that equations or interpretations developed to explain a phenomenon in one organism need not hold without modification for comparable processes in other organisms.

APPLICATION OF PHOTOGRAPHY TO SWELLING CELLS

That photography could be applied to the determination of the non-solvent volume of the eggs of *Echinometra lucunter* has been shown (Leitch, 1936), but that the photographic method can also record the actively swelling cell is indicated in Graph 2 (p. 57), in which are plotted two sets of points, one determined by measurements with a filar ocular micrometer and the other by measurements of photographs. In both series eggs from one female were used, the experiments being completed within 30 minutes from the time of spawning. The close agreement which exists between the two series of values substantiates the validity of the photographic method which has the added advantage that each point on the curve represents the average of measurements from 10 to 15 eggs, rather than being derived from measurements based upon a single egg. In table 5 the permeability constants, derived from two experiments involving measurements obtained by each method, while showing some variation, agree reasonably well.

TABLE 5—*Permeability constants of eggs of Echinometra lucunter in 60 per cent sea-water solutions, determined (a) by measurements with a filar ocular micrometer and (b) by measurements of photographs.*

Time in minutes	(a) Filar micrometer method			(b) Photographic method		
	K_2	K_4	K_6	K_2	K_4	K_6
1	0.472	0.467	0.526	0.486	0.455	0.400
2	.462	.379	.453	.421	.377	.428
3	.402	.287	.351	.368	.286	.348
4	.340	.280	.350	.359	.324	.404
5	.361	.278	.352	.362	.269	.361
6	.350	.303	.388	.340	.283	.361

EFFECT OF TIME FROM SPAWNING ON SWELLING AND SHRINKING OF EGGS

In Graphs 3 and 5 (pp. 58-59) are given the swelling and shrinking curves, respectively, for the eggs of *Echinometra lucunter* in 60 per cent sea-water for the former and for the latter in 100 per cent sea-water after the eggs had reached equilibrium in 60 per cent. The primary effect of allowing the eggs to stand after spawning is a very decided increase in the non-solvent volume from approximately 30 per cent for those eggs used not later than 1½ to 2 hours after spawning to about 53 per cent for those standing for a longer period of time (table 1). The permeability constants of equation (2) for both sets of experiments are given in table 6. In both cases there is a slight decrease in the constants with increased time after spawning, indicating that the rate of entrance and exit of water is affected as well as the equilibrium conditions. The effect is more pronounced in the case of shrinking than of swelling. The change does not appear until from 1½ to 2 hours after the time of spawning and is correlated with changes observed in the percentage development of the eggs. These observations on *Echinometra* eggs are in direct contrast to the increase in permeability of the eggs of *Arbacia* with age as observed by Goldforb (1935).

TABLE 6—*Effect of time elapsing from spawning on permeability constants of Echinometra eggs as calculated by equation (2) for swelling and shrinking experiments.*

The three columns under each heading refer as follows: A to the constants of normal eggs measured within 30 minutes after spawning; B eggs measured 2½ hours after spawning and C eggs measured 4¼ hours after spawning.

Time in minutes	Swelling experiments			Shrinking experiments		
	A	B	C	A	B	C
1	0.472	0.379	0.365	0.457	0.372	0.323
2	.462	.392	.367	.412	.321	.291
3	.402	.363	.334	.397	.297	.264
4	.490	.337	.307	.378	.288	.264
5	.361	.339	.298	.347	.289	.253
6	.350	.341	.301	.341	.287	.256

SUMMARY

1. An analysis of the equations developed to interpret the kinetics of water exchanges of cells shows that the so-called permeability "constants" are not constant for the eggs of *Echinometra lucunter* but vary with the dilutions of sea-water used and also with different intervals of time in the same dilution.

2. The permeability constants for swelling and shrinking do not coincide, being between 0.250 and 0.650 for the former process and between 0.180 and 0.580 for the latter. (These values are in terms of the number of cubic micra of water passing through 1 square micron of surface in one minute under an osmotic pressure difference of 1 atmosphere.)

3. The introduction of the correction for the non-solvent volume into the equations does not produce a better agreement between the constants for the two processes.

4. The use of the photographic method (Leitch, 1936) is further substantiated for the determination of non-solvent volumes and is extended to the study of the swelling and shrinking of eggs.

5. Approximately two hours from the time of spawning the non-solvent volume of the eggs is greatly increased, from 30 per cent to 53 per cent of the initial volume.

6. There is a slight retarding effect on the water exchanges of the eggs brought about by standing. However, this effect is overshadowed by the much greater change in the non-solvent volume.

7. The time at which the effect of standing at room temperature appears in the values of the non-solvent volume and rate of penetration of water is correlated with (a) a sharp decrease in the percentage of development, (b) a slight increase in the volume of the eggs and (c) a cytolysis-like phenomenon which finally ends with the complete disintegration of the eggs.

8. The production of fertilization membranes as a criterion of non-injury of the egg cells is shown to be inadequate and the percentage of development of normal larvæ is urged as a better test of normality.

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PAPERS FROM TORTUGAS LABORATORY, VOL. XXXI

IV

EARLY DEVELOPMENT OF PTYCHODERA BAHAMENSIS

By Fernandus Payne

With two plates

[Issued July 24, 1936.]

EARLY DEVELOPMENT OF PTYCHODERA BAHAMENSIS[1]

On account of the scarcity of complete and accurate studies on the early development of the Enteropneusta, I wish to record some observations made on *Ptychodera bahamensis* during the summer of 1933 while working at the Carnegie Laboratory at Tortugas, Florida. In only one species, *Balanoglossus clavigerus,* is the development known from fertilization to metamorphosis.

The animals were found most abundantly on the coral reef near Fort Jefferson in water which at low tide was from one to two feet deep. Eggs and sperm were obtained by placing sexually mature individuals in glass dishes free from sand. Spawning sometimes occurred in a brief time or might be delayed for a day or two. Disturbance, such as moving the animals from one dish to another, seemed to hasten spawning.

During the month of July an occasional individual spawned, but both ova and sperm were not obtained at the same time until July 31 and at intervals thereafter up to August 15. As the laboratory closed August 20, and I had had little experience in handling the larvæ I did not succeed in carrying them through to metamorphosis. This should be done to make the developmental history complete. Without such histories many errors will continue to exist in assigning tornariæ of unknown origin to specific adults.

The eggs measure about 85 microns in diameter. The yolk is uniformly distributed, giving the appearance in sections of a beautiful alveolar structure. A thick clear gelatinous membrane surrounds the ovum at the time of maturity and a fertilization membrane is formed after the entrance of the sperm. The latter, which retains its contact with the egg, is formed in large part from darkly staining particles within the cytoplasm which migrate to the periphery of the egg (fig. 3).

At the time of ovulation the first polar spindle is in metaphase. The developmental stages studied are given below in chronological order. There may be slight variations in different lots of eggs, but the time intervals are essentially the same and compare favorably with the same stages for *Balanoglossus clavigerus.*

Fertilization	11 hr. 30 min. a.m., 8, 5, '33
2 cell stage	1 hr. 05 min. p.m.
4 cell stage	1 hr. 45 min.
8 cell stage	2 hr. 20 min.
16 cell stage	3 hr. 00 min.
32 cell stage	3 hr. 40 min.
64 cell stage	4 hr. 15 min.

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[1] Contribution No. 246 from the Zoological Laboratory, Indiana University.

Blastulæ	8 hr. 15 min.	
Beginning gastrulation	9 hr. 15 min.	
Gastrulæ	12 hr. 30 min.	a.m., 8, 6, '33
Constriction of hydrocœle	8 hr. 45 min.	
Larvæ moving inside egg membranes	2 hr. 30 min.	p.m.
Free-swimming larvæ	3 hr. 30 min.	
Dorsal water pore formed	7 hr. 30 min.	p.m., 8, 6, '33
Ciliated bands present—oral and anal openings formed—gut shows three divisions—apical cilia long	11 hr. 00 min.	a.m., 8, 8, '33
Posterior ring of cilia formed—eye spots well developed—shift in position of lateral ciliated bands		8, 14, '33

The early cleavages are equal and are so nearly like those of *Balanoglossus clavigerus* as described by Stiasny (1914a) that little more need be said. The first cleavage is of interest in that the division figure is somewhat eccentric, lying nearer the animal pole, and is slightly bent. The bending becomes more pronounced in the late anaphases (fig. 2). Apparently because of the position of the division figure, or at least in correlation with it, the first cleavage furrow makes its appearance at the animal pole considerably earlier than at the vegetal (fig. 2). In this respect it is similar to the Ctenophore (*Beroë ovata*) egg, although the furrow at the animal pole cuts in only about 1/5 or 1/6 the diameter of the egg before it starts at the vegetal pole.

The blastocœle appears comparatively early but never becomes a large cavity because of the fact that the cells surrounding it are elongate (figs. 5 and 6). The yolk accumulates in the outer ends of the cells. Gastrulation is accomplished by means of a typical invagination (fig. 7). The details of the closure of the blastopore were not followed, but all indications are that the processes are the same as in *Balanoglossus clavigerus*.

The gut is formed by invagination and the closure of the blastopore. In its initial stages it is more or less spherical in shape. After the hydrocœle is constricted, the anterior end of the gut bends toward and eventually comes in contact with the ventral body wall (figs. 11 and 12). Contact with the posterior body wall has been maintained from the time of the closure of the blastopore. These changes give rise to a closed gut, smaller at the ends and enlarged in the mid-region, and with the buccal and anal regions definitely marked (figs. 11 and 12, swimming ciliated larvæ, 36 hours old). Within the next 12 hours the oral and anal openings break through and the gut becomes clearly divided into three parts, with the mid-gut largest (fig. 13). In the living larvæ the relative shapes of the subdivisions vary, due to contractility of the walls. With slight changes the shape of the gut remained about as described as long as the larvæ were kept. The walls, however, which were in the beginning made up of cells more or less cuboidal in shape, become much thinner, due to changes in size and shape of the cells (fig. 18, a reconstruction drawing made from living larvæ and sections). The inner wall of the gut is uniformly ciliated in two-day old larvæ, but in later stages distribution becomes more restricted (fig. 18).

The hydrocœle is formed as a constriction from the gut and is, at the beginning, somewhat spherical in shape (fig. 8). As differentiation proceeds, the spherical form is lost, due to an elongation in the anteroposterior direction (fig. 10). Figures 9 and 10 are drawings of the same stage but taken at right angles to each other. The cavity gradually enlarges and a dorsal extension comes in contact with the outer body wall, after which the opening to the exterior soon forms. This occurs about 36 hours after fertilization. At the same time an anterior extension of the hydrocœle makes contact with the apical plate region. With further growth this extension elongates and apparently differentiates into muscular tissue as it is contractile in older larvæ. The figures show these changes better than words can describe them. My observations, though not conclusive, indicate the dorsal opening of the hydrocœle is in the midline rather than at one side (fig. 15).

At the time the larvæ escape from the egg membranes they are uniformly ciliated, but between 36 and 47 hours the ventral, lateral, and pre-oral ciliated bands appear. Their position is best described by figures 14 and 15, lateral and dorsal views of larvæ 71½ hours old. Following the third day, there are shifts in the position of the lateral bands, best illustrated in figures 16 and 17, larvæ nine days old. A narrow posterior ciliated band is present at this time. The detailed relationships of the ciliated bands and the apical plate were not studied, although in the early stages the lateral and pre-oral bands appeared to be continuous through the apical region.

For convenience of study Stiasny-Wijnhoff and Stiasny (1926 and 1927) have recognized several different stages in the development and metamorphosis of larvæ. The stages are named after the authors who first described them and, while purely artificial, do help in assigning tornariæ of unknown history to their proper places in development. If I were to place in this scheme the oldest tornaria of *Ptychodera bahamensis* which I have observed it would approach the Metschnikoff stage. The greatest length attained by tornariæ nine days old was about 262 microns.

Stiasny-Wijnhoff and Stiasny (1927-1931) in a comprehensive survey have brought together the data about all known described tornariæ. Much of it consisted of isolated and incomplete observations here and there—in many cases only a single specimen or a single stage having been described. The authors have attempted to bring order where chaos existed and have accomplished much, but the facts are, in many instances, too fragmentary. They have assigned tornariæ to known species of Enteropneusta, in some cases because the tornaria and adult species were found in the same locality. For example, they assign the tornariæ, described by Weldon from Nassau, and those described by Morgan from North Bimini and Nassau to *Ptycho-*

dera bahamensis. They also are inclined to think that G. Stiasny's *Tornaria morgani* from Saba, West Indies, may also belong to *Ptychodera bahamensis.* Since these larvæ described by Weldon, Morgan, and Stiasny and assigned to *Ptychodera bahamensis* do not agree with my own observations of the larvæ of *Ptychodera bahamensis* which have been followed from the fertilized egg, it seems conclusive that more than one species is involved or that errors have been made on the part of some one. Even though I have not followed development through to metamorphosis, it seems clear that the tornariæ, described by Weldon and Morgan are different from the tornaria of *Ptychodera bahamensis,* assuming my identification is correct.

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PLATES

PLATE 1

FIG. 1—Egg in anaphase of first cleavage. Note eccentric position of spindle.

FIG. 2—Egg in late anaphase of first cleavage. Note early constriction at animal pole.

FIG. 3—Two cell stage—division equal. Note fertilization membrane.

FIG. 4—Four cell stage—division equal.

FIG. 5—Morula—note blastocœle.

FIG. 6—Blastula—cells elongate, slightly more so at vegetal pole.

FIG. 7—Gastrula.

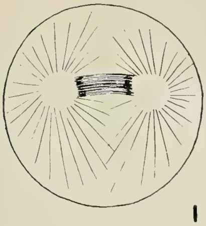
FIG. 8—Constriction of hydrocœle from gut.

FIG. 9—Hydrocœle separated from gut.

FIG. 10—Same stage as figure 9, but drawn at right angles. Note dorsal extension of hydrocœle.

FIG. 11—Gut is in contact ventrally and posteriorly with body wall indicating position of mouth and anus. Hydrocœle is in contact anteriorly and dorsally with body wall. Larva is ciliated and free swimming—36 hours old.

FIG. 12—A stage slightly later than figure 11. The dorsal water pore has formed.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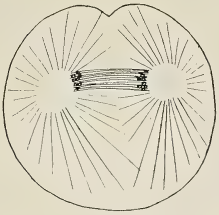
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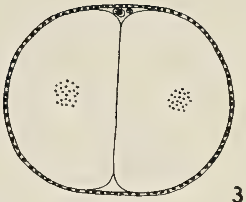
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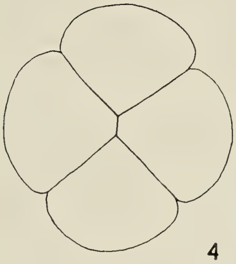
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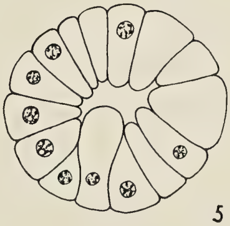
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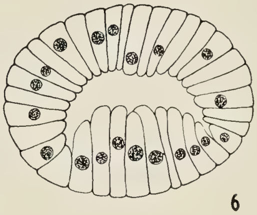
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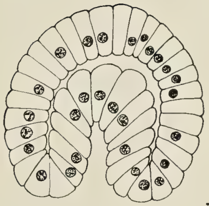
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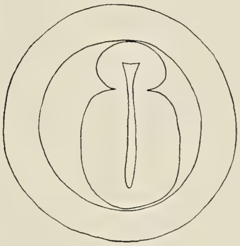
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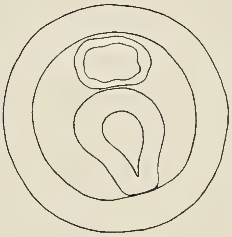
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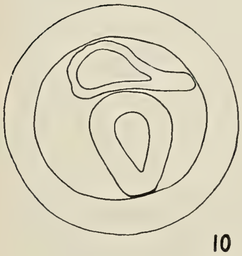
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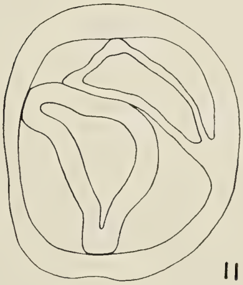
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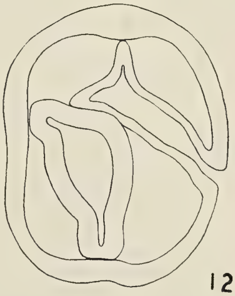
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Plate 2

Fig. 13—An outline sketch of a tornaria about 50 hours old—drawn after fixation and mounting in Canada balsam. The shape is modified slightly by the fixative. All other tornariæ with the exception of figure 18 were drawn from living specimens.

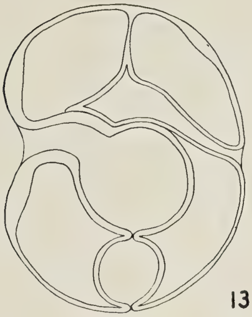
Fig. 14—Tornaria 71½ hours old—lateral view, showing position of ciliated bands, the gut, and hydrocœle.

Fig. 15—The same as 14 but drawn from dorsal surface. Note central position of hydropore. At this time irregular shaped mesenchymal cells of unknown origin are present in the perivisceral cavity.

Fig. 16—Tornaria nine days old—lateral view. A posterior ciliated band is present. There has been a shift in the location of the lateral band. The apical sense organ is more highly differentiated and the eye spots are well developed. The tissue connecting the hydrocœle and the apical region is now contractile. Length 262 microns.

Fig. 17—The same stage as figure 16 but drawn from the ventral surface.

Fig. 18—A reconstruction drawing made from living tornaria and from sections.



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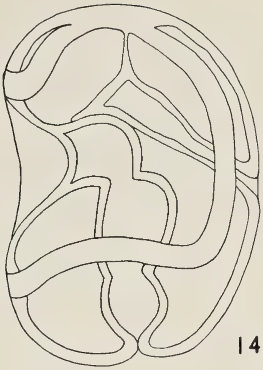

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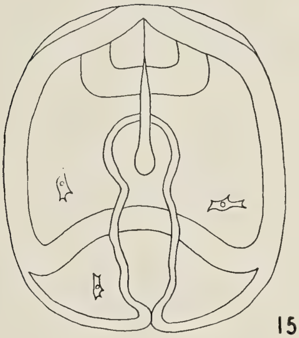

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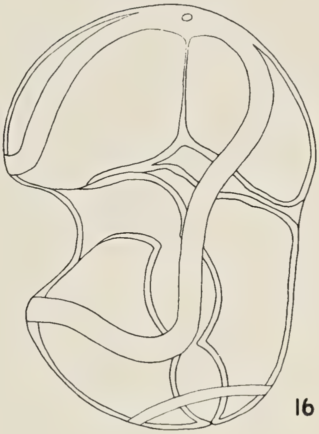

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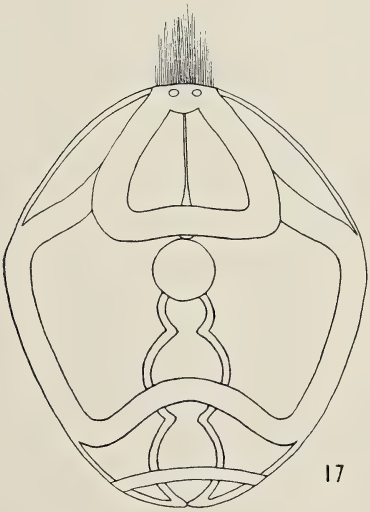

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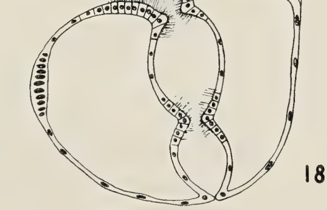

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PAPERS FROM TORTUGAS LABORATORY, VOL. XXXI

V

REACTIONS TO LIGHT IN PTYCHODERA BAHAMENSIS, SPENGEL

By Walter N. Hess

[Issued August 1, 1936]

REACTIONS TO LIGHT IN PTYCHODERA BAHAMENSIS, SPENGEL

INTRODUCTION

Except for the paper by Crozier (1917), little attention has been given to the study of light reactions in any of the Enteropneusta, and nothing is known, apparently, concerning the distribution or even the existence of photoreceptors in this important group of animals. The purpose of this investigation is to continue work on reactions to light and the photoreceptors in animals, using at this time a more highly evolved species than the earthworm on which the earlier work was done (Hess 1924, 1925). Moreover, since this animal is usually considered a member of the early Chordates, it was thought that its study might throw some light on the vertebrate organs of vision.

The author is much indebted to the Carnegie Institution of Washington for the facilities of its laboratories at Tortugas, and especially to the Executive Officer of the laboratories, Dr. William H. Longley, for his cooperation in providing every facility required for carrying on this investigation.

MATERIALS AND METHODS

Ptychodera bahamensis was used exclusively for this study. Freshly collected specimens, which were obtained near the laboratory at Tortugas, were taken at once to the dark room except as stated below. Here they were placed in crystallizing dishes that were provided with slowly flowing water, where they were dark-adapted for one hour. The animals were then transferred in turn, by means of paraffined section lifters, to a glass plate that had been covered with a mixture of paraffin and lamp black. The edges were built up so that when the animals were placed on the tray they could be nearly covered with sea-water. By replacing the water on this tray every few minutes it was possible to keep the temperature approximately constant at about 30°C.

Unless otherwise specified, the apparatus used for obtaining a source of light consisted of a 6 volt, 15 c.p. gas-filled concentrated tungsten filament lamp, which was mounted in a light-tight box containing an opening 8 x 15 mm. in size and supported in such a way as to produce a horizontal beam of light. By varying the distance of the organisms from the source of illumination, it was possible to obtain light that varied in intensity from 1453 to 0.32 meter candles.

The observations on the reactions to light of the different animals were made as follows: Each animal was lifted in turn from the crystallizing dish by means of paraffined section lifters and placed on the shallow paraffined tray described above, so that the axis of the body was perpendicular to the direction of the light rays. The shutter of the box containing the light was then opened and the reaction of the animal recorded. By revolving the paraffined tray, and at the same time controlling the shutter of the box containing the light, the right and left sides of the animals were alternately exposed to the light, which was kept uniform at the illumination specified.

The record of reactions that were made during these experiments represents only well-defined lateral movements of the anterior end of the animal except as stated below. Minor and indefinite reactions were ignored during these experiments. Each animal was studied until twelve responses were obtained, six to illumination from the right and six to illumination from the left. Due to the fact that these animals did not stand up well under laboratory conditions different animals were used for each study.

NATURE OF RESPONSE TO LIGHT IN PTYCHODERA

As reported by Crozier (1917) best results were obtained in the study of light reactions when the animals were fresh, and consequently, unless otherwise stated, animals that had been in the laboratory for two hours were discarded. The capacity of these animals to respond to light is largely determined by their previous environmental conditions. It was found that photosensitivity diminished with continual exposure to light or to the unnatural conditions of the laboratory. Animals that had been kept in the dark room of the laboratory for one hour reacted to light of a wide range of intensities, but individuals that had been exposed for a few minutes to sunlight, or to a beam of strong light, often failed to respond at all.

When the earthworm is exposed to lateral illumination as much as one-third of the anterior end of the body often moves in orientating to the light. In *Ptychodera,* on the other hand, the proboscis is usually the only part of the body that turns during the early stages of orientation, but the collar and the tip of the abdomen may also turn in response to stimulation by light. The movements of *Ptychodera,* when exposed to light, are slow and deliberate and as a rule the animal does not usually show evidence of preliminary trial and error reactions in the process of orientation. When the anterior region of the animal is exposed to lateral illumination, the proboscis usually turns directly away from the source of light. If the animal is illuminated from both sides in such a way that one side is more highly illuminated than the other, the animal will usually turn directly away from the side that is more highly illuminated. If the two sources of light are of equal intensity the animal will move between the two sources of light along a path midway between the two lights.

RESPONSE TO LOCALIZED LIGHT STIMULATION

A device for projecting a small beam of light was constructed for the purpose of ascertaining the regions of photosensitivity together with the relative sensitivity of the regions thus located (Patten, 1915). This was accomplished by placing a 2.5 volt flash light bulb, that had been connected with two dry cells, in the upper end of the tube of a compound microscope. Two wooden blocks with small circular apertures at the centers were placed in the tube of the microscope to screen out all except parallel rays of light, and the entire lower lens of the 16-mm. objective, which was used in this work, was covered with black paper except for a minute circular aperture at the center. In this way a focal point of light, approximately one thirty-second of an inch in diameter, was obtained.

Twelve animals were selected for this experiment. They were placed in turn on the black paraffined tray referred to above. After being dark-adapted for one hour, and with the room in darkness except for a dim ruby light to which the animal did not appear to respond, the light described above was used in mapping the photosensitivity of the animal. Crozier (1917) maintains that the only part of the body that is conspicuously sensitive to light is the tip of the proboscis, but the animals used in this experiment were found to be definitely sensitive to light over their entire bodies. This sensitivity is shown by definite orientations away from the light or, in the case of certain regions of the body, by localized muscular movements. By means of the pin point light, it was possible to show that the anterior tip of the proboscis, the dorsal and lateral surfaces of the proboscis, and the dorsal and lateral surfaces of the collar including its anterior edge are more sensitive to light than the other regions of the body. Of all these regions the illumination of the anterior tip of the proboscis produced greatest response. Although the entire proboscis was very sensitive to light the dorsal side was slightly more sensitive to light than the ventral side. Likewise the dorsal surface of the trunk was more sensitive to light than the ventral surface.

Although Crozier (1917) claims that the tip of the proboscis is the only part that is conspicuously sensitive to light, and that "*Ptychodera* thus illustrates the principle that an animal having an apical photosensitive spot may yet by suitable movements convert this terminal area into a bilateral sense organ," our experiments clearly show that the tip of the proboscis is not the only region that is conspicuously sensitive to light and that the photoreceptors, or other structures concerned with photosensitivity, are widely distributed over the body of the animal. They also suggest that the structures concerned with photic stimulation are more numerous or more specialized in certain regions than in others.

As is well known, there are in the anterior region of the Tornaria larva two well-defined groups of sensory cells, the eyes (Bourne, 1889; Morgan, 1894). From the data given above concerning the photosensitivity of the adult animal, it would seem that these structures must be completely lost at metamorphosis, or that, if they remain in a hidden state at the tip of the proboscis, other structures approximately as sensitive as these have developed and are widely distributed over the entire body. It may be, however, that these eyes are not concerned with reactions to light even in the larva (Ritter and Davis, 1904).

RESPONSES TO LIGHT OF DIFFERENT INTENSITIES

A summary of the results obtained in studying the responses to lateral illumination are presented in table 1. This table shows very clearly that normal Ptychoderæ react negatively to ordinary intensities of light. It further shows that the percentage of negative responses decreases and the percentage of positive responses increases as the intensity of light decreases. Many animals, such as the earthworm, show a majority of positive responses when exposed to a very low illumination and it is entirely possible that *Ptychodera* would have given similar results had we been able to use a sufficiently low illumination.

TABLE 1—*Responses to light of different intensities in normal Ptychodera after being dark adapted for one hour. Twelve responses are recorded for each animal.*

Meter candles	Number of specimens	Responses of individual animals					
		From source of light			Toward source of light		
		Light on right	Light on left	Total	Light on right	Light on left	Total
1453.	20	120	120	240	0	0	0
40.	20	120	118	238	0	2	2
1.60	20	118	114	232	2	6	8
.32	20	75	63	138	45	57	102

The results obtained in this study are in general in accord with those obtained by Hess (1924) and Prosser (1934) in their studies on the earthworm. This would suggest that the mechanism for photoreception in the two animals may be quite similar, although these animals are very different in their morphology and in their systematic position in the animal kingdom.

RESPONSES TO LIGHT AFTER OPERATIONS INVOLVING THE NERVOUS SYSTEM

Various operations were performed on different animals for the purpose of ascertaining, if possible, the relation of the nervous system to responses to light in these organisms. Freshly collected animals were selected and portions of the body of each animal were removed. Following the operation, the animals were kept for six hours in glass dishes that were provided with slowly flowing water. They were then dark-adapted for one hour, after which their reactions to light were studied, with results summarized in table 2.

Seventy animals were used for this entire study. Of these ten were used for each experiment and twelve reactions were recorded for each animal—six with the source of light on the right and six with the light on the left. The *first* of these operations involved the removal of the proboscis just anterior to the basal peduncle. In the *second* operation the ventral third of the collar was removed in order to insure the entire removal of the "ventral nerve cord" in this region. In the *third*, approximately one-third of the dorsal region of the collar was removed to insure removal of the "dorsal nerve cord" in this part of the body. In the *fourth* all of the body except the proboscis and its basal peduncle was cut off. The *fifth* involved the removal of all of the body from the proboscis, including the basal peduncle.

TABLE 2—*Responses to light after operations involving the nervous system. Ten animals were studied in each group and twelve responses were recorded for each specimen when exposed to a light of 1453 m.c.*

Portion of animal removed during operation	Responses of individual animals					
	From source of light			Toward source of light		
	Light on right	Light on left	Total	Light on right	Light on left	Total
Proboscis (not its peduncle)	56	58	114	4	2	6
Ventral third of collar	57	60	117	3	0	3
Dorsal third of collar	58	58	116	2	2	4
All except proboscis and its peduncle	60	59	119	0	1	1
All except proboscis	54	57	111	6	3	9
Proboscis and peduncle	0	0	0	0	0	0
Proboscis, peduncle and collar	0	0	0	0	0	0

Since the results obtained in the first five experiments (table 2) differ considerably from the last two experiments reported in this table we shall consider the first five experiments before taking up the last two. As will be seen by consulting table 2, the reactions to light of the animals used in the first five experiments, following the different operations, are all similar to those of normal animals in that they are predominantly negative in their reactions when exposed to a light of 1453 m.c. It is entirely possible that the slight differences obtained may be due to differences in the conditions of the animals following the different operations. These results show very clearly that the so-called central nervous system is not the controlling agent in responses to light in this animal, especially as concerns the responses of the proboscis. Moreover, the results strongly suggest that there is probably a connection within the proboscis itself between the photoreceptors, or such other structures as function in connection with photic stimulation, and the inner musculature of the organism.

The *sixth* of these experiments (table 2) involved the removal of the proboscis and the basal peduncle, the *seventh* the removal of the proboscis, basal peduncle and collar. As will be seen by consulting table 2, no orientations to light were recorded for any of these animals. And as experiment six differs from experiment one in that in the former the peduncle of the proboscis was removed with the proboscis and in the latter it was not removed, and moreover as the animal is able to react to light when it has a peduncle but is unable to react when the peduncle has been removed, it would seem that the peduncle may contain a nervous center that is definitely concerned with light reactions in this animal. On the other hand, the fact that the proboscis is able to react to light without the peduncle shows that the proboscis can respond to light independently of the central nervous system. Attention should be called to the fact that these animals with the proboscis and peduncle removed do exhibit slight localized muscular movements when exposed to light, but they are unable to turn toward or away from a source of light.

Histological studies further show that the nervous tissue is unusually well developed in the peduncle and that it is better developed in its dorsal than in its ventral region. This evidence together with that given above indicates that there is a specialized nervous region in the basal peduncle that may be a coordinating center for certain bodily movements.

Since the removal of the proboscis and peduncle also results in the removal of most of the notochord it may be that these animals have been rendered incapable of orienting to light due to the absence of the notochord rather than to any specialized nervous coordinating center which may be located in the peduncle of the proboscis.

REACTION TIME OF ISOLATED BODY SEGMENTS

Freshly collected specimens were selected and carefully cut, by means of fine scissors, into different portions as follows: proboscis without basal peduncle, proboscis with basal peduncle, proboscis with peduncle and collar, collar with peduncle, collar without peduncle, portion of the middle region of the trunk, and caudal tip of the body. These separate body segments were then allowed to stand in glass dishes provided with slowly flowing sea-water for a period of six hours. They were then dark-adapted for one hour, after which they were in turn transferred to the black paraffined tray, covered with water and exposed to a vertical light of 161 m.c. By means of a stop watch, the length of time was ascertained between the first exposure to light and the response (muscular movements).

A summary of the results obtained in the above study is given in table 3. Each figure represents the average reaction time of twenty specimens. It will be observed that the different segments of the body, exclusive of the proboscis, had an average reaction time of slightly over two seconds, while the probosces from which the basal peduncles and the rest of the body had been removed had an average reaction time of 25.23 seconds.

At first sight these results seem contradictory since the proboscis is the most sensitive region of the body. But, as is well known, *Ptychodera* possesses what is usually considered a central nervous system composed of a dorsal and ventral nerve cord which extends throughout the entire extent of the animal except the proboscis. Hence the great delay in the response of the proboscis when it has been severed from the rest of the body seems to be directly associated with the fact that this portion of the body has now been severed from the central nervous system. However, when this portion of the body finally does respond, it is able to react negatively much the same as in a normal animal. Portions of animals containing the peduncle and collar, which probably contain the most highly organized nervous regions of the body, respond most quickly of all.

TABLE 3—*Reaction time in seconds of different portions of the body of Ptychodera when exposed to light of 161 m.c. following dark adaptation for one hour. Each figure represents the average reaction time of twenty specimens.*

Proboscis without basal peduncle	Proboscis with basal peduncle	Proboscis peduncle and collar	Collar with peduncle	Collar without peduncle	Middle region of body	Caudal tip of body
25.23	2.05	1.90	1.96	2.30	2.36	2.77

Not until the nervous system of this animal is better understood will it be possible fully to explain these phenomena.

SUMMARY

1. *Ptychodera bahamensis* responds negatively to ordinary intensities of light.

2. The movements of *Ptychodera,* when exposed to light, are slow and deliberate and there is little evidence of trial and error movements.

3. The entire surface of the body is sensitive to light, the most sensitive regions being on the proboscis and collar.

4. Removal of different parts of the body involving the central nervous system caused little if any decrease in the percentage of negative responses to light.

5. The reaction time of the proboscis is greatly increased when it is removed from the rest of the animal. This is taken to indicate that the central nervous system functions to speed up responses greatly, but is not essential for responses.

6. Removal of the proboscis together with the basal peduncle makes it impossible for the animal to orient when stimulated by light. This would seem to suggest that the peduncle contains a coordinating center for certain bodily movements, or that the animal has been rendered incapable of orienting, due to the removal of that portion of the body containing most of the notochord.

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PAPERS FROM TORTUGAS LABORATORY, VOL. XXXI

VI

THE DISTRIBUTION AND PHYSIOLOGY OF VALONIA AT THE DRY TORTUGAS, WITH SPECIAL REFERENCE TO THE PROBLEM OF SALT ACCUMULATION IN PLANTS

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University of California

With two plates and four text-figures

[Issued October 21, 1937]

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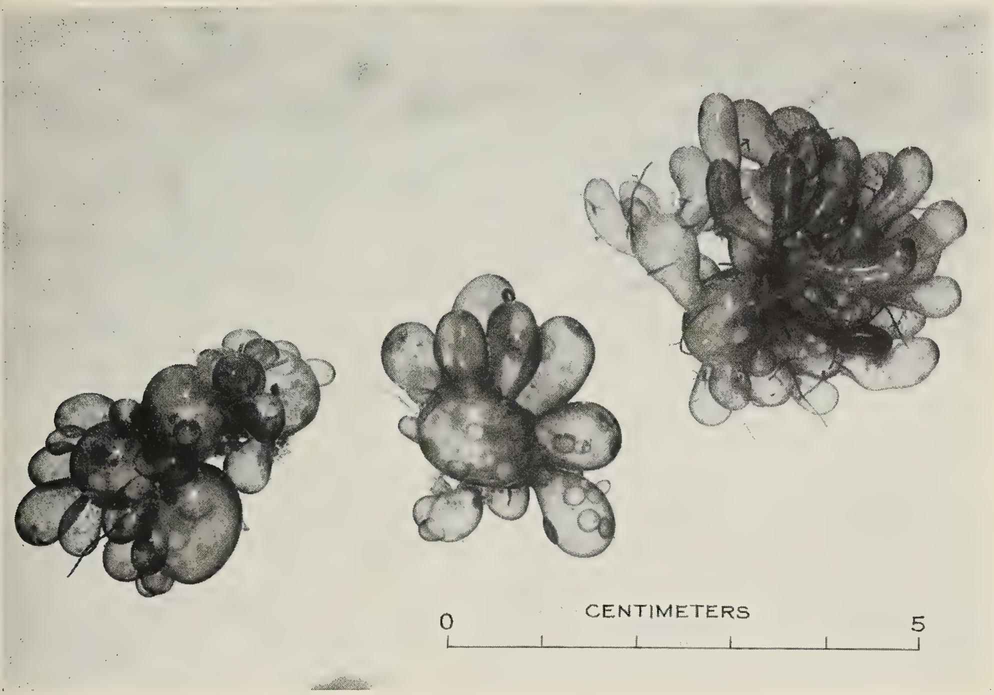
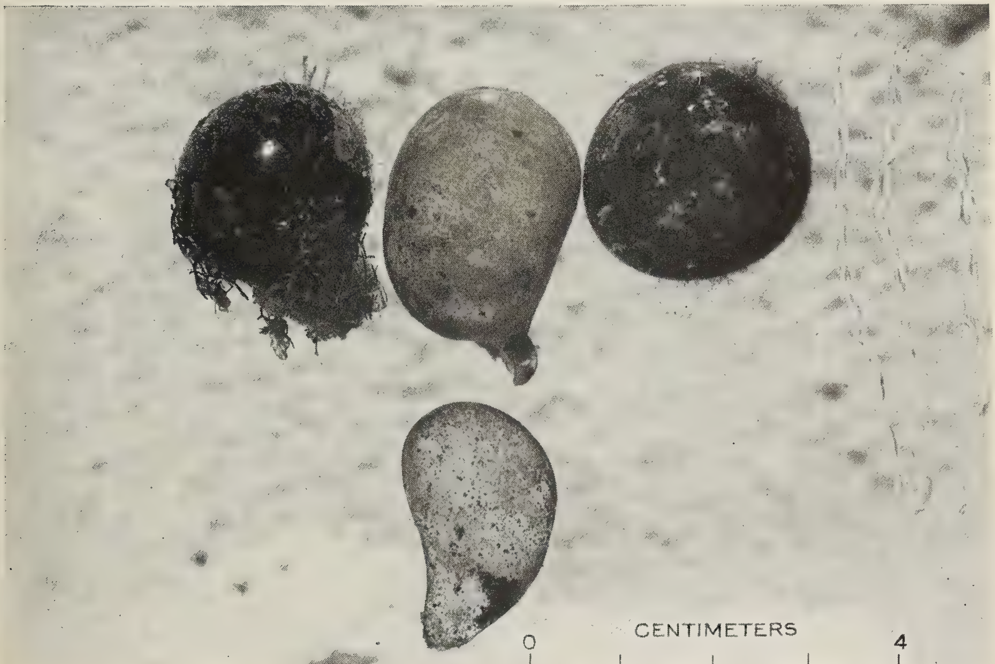
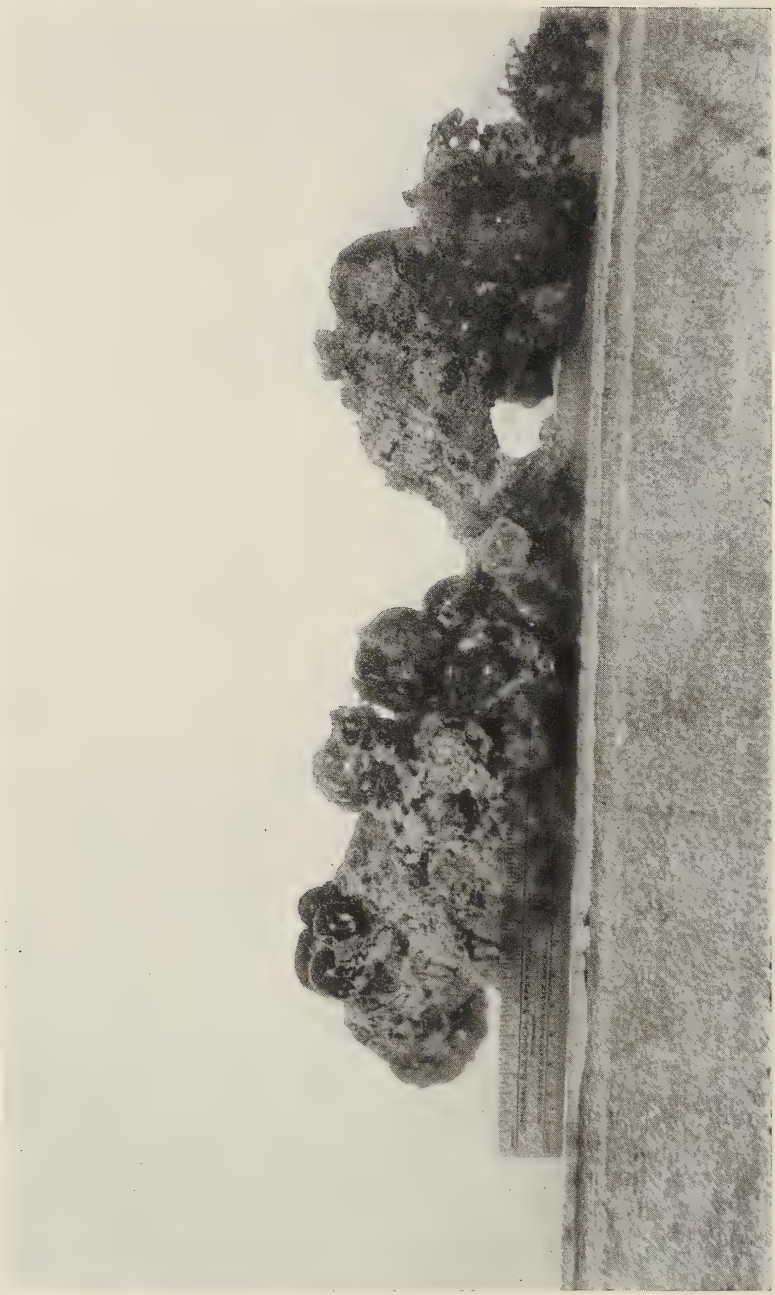


A—*Valonia macrophysa*



B—*Valonia ventricosa*



Valonia ventricosa growing on coquina rock, showing dense clusters (center) and large specimens (right)

THE DISTRIBUTION AND PHYSIOLOGY OF VALONIA AT THE DRY TORTUGAS, WITH SPECIAL REFERENCE TO THE PROBLEM OF SALT ACCUMULATION IN PLANTS

INTRODUCTION

This paper presents the results of a survey, made during the summers of 1933 and 1934,[1] of the physiological behavior of the two species of *Valonia* which are most abundant at Tortugas, Florida.

One may well ask what justification there can be for yet another paper on *Valonia.*

Since the interest of physiologists was first attracted to this genus (Meyer, 51)[2] by the unusual morphological features it presents, a volume of physiological investigation which is almost without parallel in botanical literature has been directed toward these comparatively obscure organisms. Although relatively few plant physiologists can have had direct experience with these plants in their habitat, scarcely any biologist can be unaware of the investigations referred to, since they have pervaded the literature of general physiology, as well as plant physiology, during the past quarter of a century. Whatever the legitimate claims which may be made for such attention, they are somewhat counterbalanced by the inaccessibility of *Valonia,* which has prevented that examination by a variety of investigators which is the best safeguard against overemphasis.

A considered judgment concerning the mechanism whereby plant cells absorb and accumulate[3] inorganic salts from the surrounding medium must perforce take cognizance of this very extensive literature upon *Valonia,* most of which is germane to this problem. Another approach to the same general problem (74–77, 80–82, 84) had utilized tissue derived from various plant storage organs by experimental methods especially devised for the purpose (75). These experiments, which formed a logical sequel to earlier work by Hoagland and others on *Nitella* (31–35), revealed the general lines to be followed in seeking an ultimate solution. These general conclusions, which are now being profitably extended to the cells of growing roots and other absorbing systems of plants (30, 69, 70, 80, 82) are not in harmony with the conclusions which others working with *Valonia* would apply to plant cells in general (58, 59). In this impasse a direct examination of *Valonia* was imperative. The original purpose of this examination was to provide a basis for a physiological comparison between the principal species of *Valonia* and other organisms with which the problem of salt accumulation had been profitably investigated. All the work which this entailed will not be published here. Some of the conclusions which emerged have already been utilized in a general survey of the present status of the problem of salt accumulation by plant cells (80). However, in the course of the work it became evident that, despite the volume of highly specialized research referred to, much of the information necessary to define the physiological potentialities and limitations of this genus was still either fragmentary or lacking. Useful information of this character emerged during 1933, and these facts, which were confirmed and extended during the authors' collaboration in 1934 and since that date, will now be presented.

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[1] During 1933 the senior author worked alone. Many of the useful results were repeated in 1934, and since then much analytical work has been undertaken by the second author with his associates in the Division of Plant Nutrition, University of California, Berkeley, California.

[2] From Meyer's modest phrase, "Eine makrochemische Untersuchung dieses Zellsaftes und ein makrochemisches Studium der Veränderungen, welche der Vacuoleninhalt von *Valonia* voraussichtlich bei Aenderung der chemischen Zusammensetzung des Aussenmediums und physikalischer Factoren zeigen wird, scheint mir für manche physiologische Fragen nicht ohne Interesse zu sein," may be seen the beginning of the extensive physiological literature on *Valonia.*

[3] Used in the technical sense throughout this paper to imply an internal concentration which exceeds the external.

A logical prelude to any discussion of the changes which occur in the sap composition of *Valonia* under experimental conditions must be an attempt to characterize the range of variation which it exhibits in nature and, if possible, the factors responsible. Despite some casual references [1] to the fact that the sap varies with the collection and with the size [2] of the vesicle, it is more frequently stressed that this fluid tends to be constant during growth (57, p. 297; 67, p. 667; etc.) and can be represented by figures which are freely stated in a precise form and referred to in tables of the chemical analysis of the sap [3] of different species (58, 59, 17, 21). These figures, which are almost invariably the results of analysis of single collections of a composite sap sample from a batch of cells, have formed the basis for the preparation of the so-called "artificial sap" which has been used to replace "normal sap" in electrical experiments (24, 63, 47). In the electrical experiments single vesicles are commonly involved, and the implication is that the "artificial sap" is identical with the sap they contain. In fact, in an oft-cited experiment (24, 63) the residual electrical properties which appeared when *Valonia* was placed in "artificial sap" are commonly referred to as evidence that the inner and outer protoplasmic surfaces cannot be identical, since they still exhibit such differences when in contact with apparently identical fluids. Such an experiment implies a complete faith in the ability to reproduce at will the composition of the fluid present in any vesicle of *Valonia*.

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[1] Blinks and Jacques (7) merely state that the K:Na ratio in the sap of *V. macrophysa* at Bermuda may vary from 2.55 to 5.72, but give no further detail. The same statement was repeated by Osterhout (59), and Höber and Höber (37) say that the Mediterranean material varied with the collection, etc.

[2] Jacques and Osterhout (42, p. 302) showed that two similar, selected batches of *V. macrophysa* which contained vesicles of three different sizes and which yielded about 12 cc. of sap did not differ by more than the errors of analysis (less than 1 per cent). A sample composed only of large "cells" (0.75–0.40 cc.) had lower potassium concentration and greater sodium concentration than one composed of small "cells" (0.40–0.20 cc.).

[3] Since the preparation of this paper a further review by Osterhout has appeared (Bot. Rev., vol. 2, 283–315, 1936). This embodies many of the figures, tables, and arguments in other reviews (58, 59) and will not be referred to further.

Further, it has been suggested that the sap composition of these large structures may have value as a taxonomic character. This view, to which Brooks gave prominence (17), arose originally, no doubt, from the familiar fact that *V. ventricosa* is commonly richer in potassium than *V. macrophysa*, a closely related species. However, as far as the authors are aware, no collections of these two organisms had previously been made from sites where they were growing side by side. Failing this, the possibility (remote, perhaps, but real) remains that the differences observed are not inherently characteristic of the organisms, but reflect the different conditions under which they commonly grow. Brooks also cited (*loc. cit.*) his own and other data for *Valonia* species, again derived, it appears, from single collections of the composite sap from plants growing in habitats as far separated as the Dry Tortugas, Bermuda, Naples, and Tonga. The implication is that the single analysis represents the sap composition of the *Valonia* species in question and that the different species may be characterized by reference to these scanty data, no two of which are identical. If one may judge by the frequent repetition of the same figures, the composition of the sap of *V. macrophysa* at Bermuda is constant. The figures in question have been repeated in at least the following papers: 58, 59, 55, 57, 54, 64, 21, 17.[1] They were derived originally from a collection of sap[2] made by Crozier and submitted in 1919 to Van der Pyl for analysis. Since then these figures have been accepted without question as representative of the *V. macrophysa* as it exists at Bermuda.

With respect to the sap composition of species in the genus *Halicystis* a similar situation prevails. *V. ventricosa* (Bermuda, floating specimens) was for long contrasted with *V. macrophysa* (64). This comparison was prominently cited because it apparently showed two closely related species one of which stored potassium as the principal cation and the other sodium. When it was subsequently realized that the floating specimens in question belonged to the genus *Halicystis* (5), they were eventually designated *Halicystis Osterhoutii* (6), and their sap composition, again defined by a scanty number of analyses from a few collections,[3] is now frequently contrasted with that of *H. ovalis* from the Pacific Coast, because the latter species has a higher potassium content and a lower sodium content than the former (7, 59, 39, 14).

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[1] Also Bot. Rev., vol. 2, 283–315, 1936.

[2] Three hundred cc. of sap from plants merely rinsed, wiped dry, and the sap extracted by gentle pressure from the pierced vesicles. The other published sap analyses from Bermuda seem to have been made on material which has been dissected and allowed to adjust for some time to diffuse light and sea water in glass containers.

[3] Twenty-five cc. of sap from 300 "cells" in three collections.

Unfortunately localities as well as species have been compared on the basis of very few determinations of the sap composition of species common to them. Cooper and Blinks (21) reported one set of figures which, it was stated, referred to "freshly collected cells" of *V. macrophysa* from the moat of Fort Jefferson. Presumably because the analysis obtained differed somewhat from the original data from Bermuda (54), which are invariably the standard by which this species is judged, Blinks concluded that the *V. macrophysa* of Tortugas is "not healthy," and "contamination" was suspected "by the entrance of the ions of sea water." To this argument must be traced, apparently, the origin of that frequent imputation that the Dry Tortugas compare unfavorably with Bermuda for the investigation of *Valonia*. This regrettable aspect of the subject will not be discussed at this point except to remark that the futility of such evidence and conclusions will be revealed when the relevant data are presented.

At the outset of this investigation the authors were prepared to assume that the sap composition of *Valonia*, in contact with sea water unmodified as to its composition, would have a degree of fixity which, as the work proceeded, proved to be illusory. This raised the whole question of the so-called "normal sap composition" of *Valonia*, and this must now be reexamined.

Before we embark upon this task it may be well to inquire why the sap composition of *Valonia* species should ever have been accredited with a constancy which would not commonly be attributed to any plant, even though unusually uniform in genetic composition, growing in its variable natural habitat. It is true that the sea is a much more constant external environment than most fresh-water or land organisms encounter. Even so, important variations in oxygen content and acidity occur (53, 73),[1] especially in tide pools and the more isolated bodies of water, in which the marine flora is commonly rich. However, light and temperature, so often evident as determining factors in the total distribution of the flora, may also be expected to influence its content of inorganic salts. It is significant that the prevailing mechanistic outlook upon the study of *Valonia* has been so preoccupied with those external factors ([K], [Na], [H], [OH]) which remain relatively constant that little regard has been given to any approach to the problem which recognizes that other variables, which operate through the vital processes of the plant during its growth, may determine the composition of its sap. An unfortunate result of the prevalent impression that the sap composition is constant is that investigators have not appreciated fully the value of those natural experiments which are available whenever differences of sap composition can be correlated with habitat factors. Some observations in this paper will indicate the possibilities in this direction.

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[1] See also papers by McClendon in Carnegie Inst. Wash. Pubs. Nos. 132, 1911; 183, 1914; 251, 1917; 252, 1918.

I. THE CHARACTERISTICS AND OCCURRENCE OF VALONIA AT TORTUGAS

Three valid species of *Valonia* grow at Tortugas and are readily accessible in sufficient quantity to permit their use in physiological experiments. These are *V. macrophysa* Kuetzing; *V. ocellata* Howe; and *V. ventricosa* J. Agardh. The necessary taxonomic authority may be obtained from Taylor (86, pp. 74–75). *V. ocellata,* though it is quite abundant on Long Key and Bird Key Reef and in the moat of Fort Jefferson and was frequently encountered during the collection of *V. ventricosa,* has not received the physiological attention which it deserves, no doubt because of the smaller size of the vesicles produced. As in previous investigations, so in this, all attention has been confined to *V. macrophysa* and *V. ventricosa.*

The morphological features of these two species are distinctive. *V. macrophysa* (see Plate 1A) branches freely and forms a close, palisade-like mass. The branches are cut off from the "parent" vesicle by the curved circular septa, the *Uhrglaswand* described by Kuckuck (49) and Oltmanns (52).

Valonia ventricosa (see Plate 1B), however, does not branch. When groups of plants occur, and this happens frequently (see Plate 2), they are usually distinct, or if in close clusters they readily separate, or at most adhere by the minute rhizoids and hapteron cells and are not in organic connection. No dissection of any kind is, therefore, necessary with this species; the entire plant may be used as it is removed from the substratum.

In physiological work the large, multinucleate vesicle is commonly designated a single cell, and it is tacitly assumed that the cavity filled with sap is homologous with the vacuole of the parenchyma of flowering plants. Bailey and Zirkle by staining methods (2) distinguished at least two types of vacuoles which are widespread. They designated these types A and B. Since they state that the large central vacuole of *Valonia* is of type B, they imply that it is comparable with the smaller vacuoles included within the protoplast of flowering plants. A full discussion of the questions which this raises is not possible here. However, any who have collected *V. ventricosa* at Tortugas cannot but be struck by that other member of the Valoniaceæ, *Dictyosphæria,* which is also abundant. This plant encloses within an outer sheath of segments cut off by septa (which in *Valonia* are represented by the inconspicuous marginal cells) a central cavity which in young specimens may contain solution, later both gas and liquid, and in old broken specimens becomes continuous with sea water. Clearly in this case, as probably also in *Valonia* despite the absence of septa, the vesicle is not a single cell, and the fluid contained in it should be compared, not to a single vacuole, as for example in a parenchyma cell, but rather to an internal fluid surrounded by a sheath of living cells, as for example the fluid contained within the stele of a vascular plant. It is not impossible that a cytological examination by the methods now applied to the vacuome would reveal inclusions which are the true homologues of the vacuole. Doyle's observations (25), so far as they go, show that vacuoles are present in the cytoplasm, and he believes that the large "vacuole" is formed when several coalesce. This may seem a trivial point, but in the case of a "type" organism from which generalizations have been freely extended to cells in general it assumes considerable importance. It is true that the phenomena of salt accumulation with which the work on *Valonia* has been primarily concerned may occur in intercellular as well as intracellular fluids, for example that contained in the stele of higher plants (80, and references there cited). Brooks (17) believes that accumulation occurs in an intercellular cavity in *Codium*. Clearly, however, this accumulation does not arise there de novo, but involves the intervention of active living cells which release their accumulated salts into a fluid which is enclosed by living cells but is itself not essentially part of the living system. Brooks (19) also claims that high concentrations of rubidium may occur in the protoplasm of *V. ventricosa* and that the rubidium is subsequently secreted into the central cavity. The idea is to be commended, but the evidence is indirect and is based only upon certain discrepancies between the total amounts of rubidium found and expected at the end of experiments. Since Brooks himself regards the results as provisional, little weight can be attached to them till it is conclusively established that they were not due solely to the analytical procedure. It is a curious fact that the thinner-walled secondary rhizoidal cells (27, 86) or hapteron cells which develop from the frequent marginal cells of the *Valonia* vesicle have never been considered from this point of view. These are numerous (e.g. 200 on a vesicle of 1 cc. volume), and there is also the suggestive fact observed by Doyle (25) that active protoplasmic streaming (a property often associated with the capacity to accumulate salts [4])may be observed in these cells but not in the large vesicle.[1] The theories of salt absorption by *Valonia* and the large number of electrical experiments upon which they are in part based have merely assumed that the salts are absorbed over the whole surface of the vesicle. In view of the structures referred to, this tacit assumption needs to be examined.

The only cytological examination of the large vesicle seems to be that of Doyle (25). Other than the points already referred to, the important observations made were as follows: Over 65 per cent of the total cytoplasm is composed of plastids which are plentifully supplied with starch grains (8μ to 3μ in size). Hollenberg (40) also found starch in *Halicystis*. In addition to starch, food storage in *Valonia* is also in the form of granules designated lipoid by Doyle. The clear cytoplasm is confined to a diffuse network around its bulky inclusions. Division of the very numerous nuclei

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[1] Van Iterson (Nature, vol. 138, 364, 1936) suggests that protoplasmic streaming may determine the direction of the glucose chains in the wall. This hypothesis does not appear to be based on observations of streaming in the large vesicle.

(300 per sq. mm.) tends to maintain a constant number per unit of surface. Other sources (71) may be consulted for the nuclear behavior in connection with reproduction.

The outstanding facts are that *Valonia*, contrary to opinions frequently expressed, which are apparently based entirely on the examination of the sap of the central cavity, is plentifully supplied with storage products. The cytoplasm is far from a simple homogeneous phase, and the cytoplasmic organization presents still further difficulty in the interpretation of the numerous electrical experiments which have been made on *Valonia*. In view of this and the still open controversy regarding the true nature of the E.M.F.'s recorded on these plants (50, 58), no further reference will be made to the extensive literature dealing with the bioelectric phenomena of *Valonia*.

In both cases (*V. macrophysa* and *V. ventricosa*) the wall is of cellulose, and, despite the difference in habit, the orientation of the glucose chains in space seems to be identical.[1] The wall is laid down in laminæ which can be separated by careful manipulation without rupturing the turgid cells. From *V. ventricosa* these laminæ can easily be removed in long films thin enough to show interference effects. Even these, it seems, may be composed of still thinner laminæ,[2] since they, like the whole wall (1, 69*a*, 72) have cellulose "fibrils" laid down in two directions. According to Preston and Astbury (69*a*), the wall is laid down in alternating layers. In one set of layers the glucose chains form a system of "meridians" and in the other a "loose" spiral which "closes down" upon the "poles," one of which is found in the region of the rhizoids. Casual observation on *V. macrophysa* suggested that the outer laminæ, which in this plant comprise a wall thinner than that of *V. ventricosa,* may be continuous over the surface of both the "parent" cell and those branched from it. The smaller vesicles of *V. ventricosa* especially are extremely turgid, though the wall still retains its elastic properties, as the almost spherical structures will rebound from the smooth surface of an enamel pail, and when poised in sea water, which is only slightly less dense than the cell sap, they rebound freely from a glass surface and must in consequence be transferred from vessel to vessel with every care. Hollenberg also remarks upon the much greater elasticity of the wall of the vesicle of *Halicystis* (40, p. 785). These incidental observations have their significance, because it has been suggested, wholly without evidence, that the wall of *Valonia* has reached its elastic limit and further extension can occur only if the conditions permit cellulose synthesis.[1] Not the least fascinating aspect of the wall of *Valonia* is the silky, iridescent appearance it presents when the organism is found free from calcareous deposits and epiphytic plants and is directly illuminated by bright sunlight.

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[1] See Astbury et al. (1) for *V. ventricosa.* Information concerning *V. macrophysa* from Dr. R. D. Preston, Department of Botany, University of Leeds, also see 69*a*.

[2] X-ray examination of strips supplied by one of us (F. C. S.) by Dr. R. D. Preston, Department of Botany, University of Leeds. Results privately communicated.

The two organisms occur in different habitats. Hitherto *V. macrophysa* has been collected at Tortugas only in the protected environment provided by the tide-filled moat of old Fort Jefferson, and *V. ventricosa* only upon the reef.

Valonia macrophysa flourishes on a horizontal, flat surface and attains its greatest luxuriance on a ledge, about a foot wide, which is borne upon the inner face of the sea wall of the moat at a level such that, for most of its length, it is exposed only at the lowest tides. The preference for this ledge is probably not unconnected with the flat, "palisade-like" mats which form the most luxuriant stands of this organism. Some detailed consideration will be given to the various stations from which *V. macrophysa* has been obtained. It may be mentioned here, however, that it is much more abundant upon the ledge mentioned than upon the vertical inner surface of the confining wall of the moat or the steeply inclined face of the fort wall, which bears no such ledge. Apparently almost all physiological work done at Tortugas has utilized cells from the position in which the plant is most accessible, namely the northern end of wall III of the fort according to Taylor's convention (see fig. 1 and 86, p. 23). Though not so accessible, it is also especially abundant on the ledge of wall VI. In varying amounts this plant may be found throughout the moat wherever the confining wall is not undermined.[2] It will subsequently appear that the different parts of the moat are associated with morphological and physiological features recognizable in the *V. macrophysa* they support. Despite a survey by one of us (F. C. S.) and inquiry from other investigators who have collected

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[1] M. M. Brooks (11) states that dissected cells of *V. macrophysa* expanded as fast as "the necessity for building new wall material permits."

Jacques and Osterhout (44, p. 744) state that "the failure to grow is due to the inability of the cell to manufacture cellulose in the dark. This might prevent the stretching of the cell wall and so render growth impossible. This is the more likely since there is little or no storage of carbohydrate in these cells. It might be supposed that in the absence of growth potassium and sodium would continue to enter. If this occurred the osmotic pressure would rise and put an increasing pressure on the cell wall and this might be expected to check the further entry of electrolytes." This can hardly be harmonized with the statement (57, p. 297) that "under normal conditions water will enter until the internal pressure nearly equals that outside as the cell wall is too elastic to offer much resistance." Since the first reference to the part played by the wall in the salt-and-water relations of *Valonia* by Wodehouse, who said (87, p. 458), " . . . this shows that salts can pass more or less freely through the cell wall and yet it offers sufficient obstruction to enable it to act as an osmotic membrane," the allusions to the wall have been vague, casual, and often misleading. There is not a single *proof* that cellulose synthesis ever limits the expansion of *Valonia,* or that cellulose synthesis in the dark is immediately limited by lack of carbohydrate.

[2] Subsequently to 1934 the moat has filled considerably. Not only has the "ledge" become somewhat broken by falling masonry in certain places (e.g. wall III), but the effects of stagnation and silt are much more evident. In consequence, places in which, in 1933–1934, *V. macrophysa* was very abundant (e.g. walls III and VI) in 1937 supported only a much more sparse growth.

Valonia at Tortugas in the past few years, there is no evidence that *V. macrophysa* ever occurs there outside the moat of Fort Jefferson. Taylor, whose careful survey of the moat and its environs was made in 1924 and 1925, describes in detail the distribution of the plants he encountered but makes no reference to *V. macrophysa* except in the moat. It is evident that *V. macrophysa* growing upon the surface of the ledge receives the direct sunlight of these southerly latitudes except when shaded by the surrounding masonry (the adjacent sea wall or the lofty but more distant wall of the fort). If stands of *V. macrophysa* become well established they cover the substratum, and so they are not often screened by more luxuriant vegetation (e.g. *Halimeda*).

Hitherto *V. ventricosa* has been collected at Tortugas only upon the reef. Apparently all the material for physiological purposes has been collected upon Bird Key Reef or the almost continuous Bush Key Reef. Here the only protection is that provided by the interstices of the coral and the hollowed cavities of the coquina rock upon which it commonly grows. In sufficiently stable tide pools having a substratum of coral fragments or the "sand" of the calcareous *Halimeda* scales, it may be found growing comparatively unprotected and fully exposed to the sun. In such pools, which were never dry even at the lowest tides, situated at the seaward edge of Bird Key Reef (see p. 126), plants have on occasion been collected in considerable number. In this kind of habitat the plants are comparatively free from incrustations of calcareous material or even other algæ, which so often occur elsewhere. This organism is, however, much more abundant in the slight protection provided by the hollowed cavities of coquina and coral fragments. In 1937 *V. ventricosa* was found in exceptional abundance in a reef pool situated at the inner edge of Bush Key (see Plate 2). A curious illusion has arisen that *Valonia* grows under conditions of extreme shade, so that some have even questioned whether it absorbs carbon dioxide by photosynthesis (13, 8). It is true that often a convenient method of collection is to roll over the bulky masses of substratum and thus expose the cavities beneath. It is very evident, however, to any experienced collector that *V. ventricosa* is never abundant, in fact rarely found, except on surfaces already colonized by other algæ, and when it is found growing underneath rocky fragments it is invariably beneath protecting ledges and cavities which, though they may screen off the direct rays of the sun, do not prevent illumination by light of high intensity reflected or scattered from the white surface beneath. During the course of the work very high light intensities have been recorded photoelectrically adjacent to both *V. macrophysa* and *V. ventricosa* in their normal habitat. The fallacy that *Valonia* will not tolerate bright light may have been aggravated by a common experience that collected specimens in glass bottles are readily killed in bright sunlight. It seems that the greater prevalence of *V. ventricosa* underneath the surfaces described is due to the mechanical protection they provide rather than to shade.

During the season of 1934 especial effort was made to secure collections from other localities than Bird Key Reef. *V. ventricosa* was observed growing fairly abundantly on the outer sea wall of the fort on Garden Key.

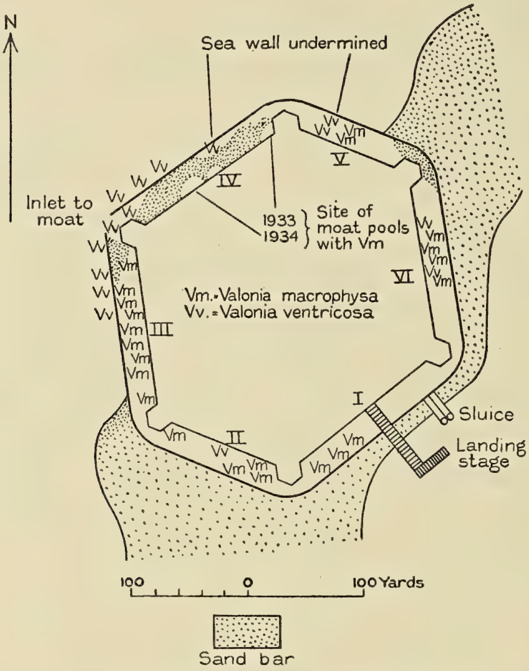

FIG. 1—*Valonia* in the moat of Fort Jefferson. (Outline drawn to scale from inset of Tortugas Harbor, chart of the Dry Tortugas, U. S. Coast and Geodetic Survey, No. 585.)

These collections were confined to walls III and IV (see fig. 1) near to the inlet. In this habitat the plants grew fully exposed to light, and it is of some interest, since (as far as we are aware) previous collections had not been made from this site, that large vesicles occurred more frequently than on the frequently collected zone of Bird Key Reef. Taylor recorded the flora of the outer surface of the westerly walls III and IV in 1924. If *V. ventricosa*, which was not listed, had occurred then as abundantly as in 1934 it is improbable that it would have been overlooked by so careful an observer.[1] This site provides the interesting juxtaposition of *V. ventricosa* (without *V. macrophysa*) growing on the outer seaward surface of the confining wall of the moat, and on the ledge of the inner surface of the same wall (merely separated from it by the four to five feet of masonry), a population of *V. macrophysa* without any trace of *V. ventricosa*. A more striking example of the subtle physiological differences which control the growth of these organisms in their habitat could hardly be desired. Taylor recorded the presence of *V. ventricosa* on the rocky masses to the northwest of the laboratory wharf on Loggerhead Key. No collections were made from this site in 1933–1934, as this plant did not occur there in abundance. However, in 1934 a rich stand of *V. ventricosa* was observed on the submerged masonry of the laboratory wharf. Though somewhat shaded by the superstructure, these plants, like those on Garden Key, were fully illuminated at some period of the day.

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[1] It is doubtful whether this fact does more than place an upper limit upon the age of the *Valonia* plants here encountered. In 1937 *V. ventricosa* was still present but consisted of rather small specimens. Since all the large ones seen were removed in 1934, this may imply that several years are required for the growth of the largest vesicles.

The most interesting new point which emerged in 1934 (83) was that *V. ventricosa* undoubtedly occurred in the moat, though hitherto unrecorded there. It may be assumed with some certainty that if this plant had occurred there in 1924–1925 it would not have escaped the careful scrutiny of Taylor. The suspicion that *V. ventricosa* was invading the moat was aroused by an unmistakable but dead specimen attached to the "sand" in a pool bounded by the east bastion of wall V (fig. 1). A collection of quite typical large, living material followed, and this inspired a careful search of both sides of the moat. For this a skiff was indispensable. Though this species could not be described as abundant in 1934, it was collected without difficulty in situations to be specified (see fig. 1), and vesicles ranging from a few cc. up to 20 cc. in volume were collected.[1]

Figure 1 shows a plan of the moat, from which the structural changes which have occurred since Taylor's survey may be judged. The outer sea wall of side IV was being undermined in 1924–1925 and the surf had elevated a sand bar which even then isolated certain pools on this side. In the interim this continued so that in 1933 there was a deep tide-filled pool of long standing sheltered by the northeast bastion and without free access to the main body of water. This pool (referred to as moat pool of 1933, fig. 1) supported a dense population of *Caulerpa* and fish of considerable size, and maintained a fairly considerable growth of *V. macrophysa*, the special features of which will be referred to later (p. 115). During the winter of 1933–1934 this pool disappeared beneath a high mound of coral fragments, and side IV was now reduced to two small tide-filled pools cut off from the moat and a rather shallow area which reproduced the conditions which obtain outside the sea wall and in which *V. ventricosa* occurred; whereas *V. macrophysa* was found in the isolated pools of 1934 and reproduced the special features of the plants from the moat pool of 1933. Wall III still remained sound and there was no free flow and surge of the surf beneath it. On this side the main body of water remained deep and was not broken into isolated pools, though it was almost completely isolated by a sand bar, except at the highest tides, from the western inlet. The tidal fluctuation was almost entirely determined by entry of water at wall I. There was no *V. ventricosa* in this arm of the moat, though *V. macrophysa* could be found abundantly on the confining wall and sparsely on the fort wall. The outer walls II, I, and VI are not exposed to surf and still remained intact in 1934, and on these sides there were no isolated pools; the sluice (wall I) remained the only contact with the open sea. Wall V had suffered drastic change. At its northwestern extremity there was in 1934 fairly free access of the surf under the much undermined sea wall. On this side the moat had filled considerably and connection with side VI and most of side IV had been completely severed by sand bars. *V. ventricosa,* which occurred plentifully on the outer surface of walls III and IV, had invaded the inner surface of the confining wall in the neighborhood of the inlet and where the sea wall was undermined on sides IV and V, but could not be found either in the large protected body of water on side III or in the isolated pools on side IV in which *V. macrophysa* occurred. Along side V the characteristic luxuriant flora described by Taylor had been supplanted by one more typical of the present conditions outside the moat. *V. ventricosa* plants were collected both from the inner sea wall and from the fort wall V, and a most interesting collection (p. 102) of *V. macrophysa,* to which later reference will be made, was obtained from the fort wall. All other collections of typical *V. ventricosa* obtained in the moat were derived from the inclined wall of the fort along the sides numbered II and VI. There is every reason to believe that *V. ventricosa* would occur on the fort wall I, but a detailed examination could not be made in 1934.

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[1] A cursory examination of these sites in 1937 indicated that large specimens were much less abundant then than in 1934, when all the large specimens seen were removed.

The interesting point is that the observed distribution of *V. ventricosa* was complementary to that of *V. macrophysa.* The latter still retained its greatest luxuriance on the flat shelf of the confining walls III and VI at places where the masonry was not undermined, so that there was not a free connection with the open sea. In such places pure stands of *V. macrophysa* uncontaminated with *V. ventricosa* were always obtained. Wherever the confining wall had become undermined and permitted beneath it a communication between sea and moat which allowed the free flow and surge of surf, the *V. macrophysa* disappeared and a sparse growth of *V. ventricosa* appeared, even upon the inner surface of the confining wall which was formerly heavily colonized by *V. macrophysa* (walls IV and V). Only in a very few sites (see fig. 1 and table 1) in the moat of Fort Jefferson did the two forms exist side by side, and these were borderline stations for both species, as the growth was sparse. Collections of *V. ventricosa* were made from the eroded cracks of the inclined surface of the fort wall, which also supported for most of its length rather infrequent and somewhat abnormal growths of *V. macrophysa.* The two species retained the characteristic features of their sap composition even when they existed in close proximity. In fact it will be shown later that *V. ventricosa* had an even higher potassium concentration when it occurred in the moat near to *V. macrophysa* than on the reef where *V. macrophysa* did not occur. The sap composition is not evidence that the two forms are distinct, since this, like the growth form, might be

TABLE 1—*Summary of the occurrence of Valonia ventricosa and V. macrophysa at Tortugas*

Site	Substratum	Conditions	*Valonia* species present
Loggerhead Key	Vertical plane surfaces of the pylons of the wharf	Fully exposed to surf	*V. ventricosa* but not *V. macrophysa*
Bird Key Reef	Interstices of coquina rock; in sheltered tide pools on *Halimeda* "sand"	Slight protection afforded by coral and coquina	*V. ventricosa* but not *V. macrophysa*
Garden Key	Interstices of eroded surface of outer vertical sea walls III and IV of Fort Jefferson	Exposed to the surf in an area sheltered by the proximity of the fort and Garden Key	*V. ventricosa* but not *V. macrophysa*
Garden Key	Inner vertical surface of sea wall of Fort Jefferson near western inlet and large gaps in wall IV	Exposed to the surf in an area sheltered by the proximity of the fort and Garden Key	*V. ventricosa* but not *V. macrophysa*
Garden Key	Inner surface of the undermined but not broken sea wall V	More protected by sea wall than similar location of wall IV	Small amount of *V. ventricosa* accompanied by sparse growth of *V. macrophysa*
Garden Key	Eroded crevices of the nearly vertical fort wall on sides V, VI, II not heavily colonized by other algæ	Not exposed to the surf	Occasional specimens of *V. ventricosa* accompanied by a sparse, irregular growth of *V. macrophysa*
Garden Key	Inclined surface of fort wall III	Completely sheltered from surf. This arm of the moat is subject to considerable diurnal fluctuations in O_2, CO_2, and acidity.	Sparse growth of *V. macrophysa* but no *V. ventricosa*
Garden Key	Horizontal ledge on inner sea wall of sides VI, I, II	Completely sheltered from surf in vicinity of rich algal flora and subject to diurnal fluctuations of O_2, CO_2, and acidity	Exclusively *V. macrophysa;* especially luxuriant on wall VI
Garden Key	Inner horizontal ledge of sea wall III	Completely sheltered from surf in vicinity of rich algal flora and subject to diurnal fluctuations of O_2, CO_2, and acidity	Very rich stands of *V. macrophysa* but no *V. ventricosa*
Garden Key	Coral fragments in small isolated tide pools along fort wall IV	Completely sheltered from surf and subject to more extreme diurnal fluctuation of O_2, CO_2, and acidity	Irregular growth of *V. macrophysa* but no *V. ventricosa*

determined by factors which operate upon an "all or none" principle and do not permit a graded series of intermediates. A suggestive fact is that the species are indistinguishable by their chloride content (table 4) and differ only in the proportion of potassium to sodium, a relation which is most sensitive to environmental changes. Only one location (fort wall V) produced material which was described when collected as transitional in form,[1] and it is an interesting fact that these samples, though designated *V. macrophysa,* had unusually high potassium and low sodium concentration for this species, namely [K] = 0.560 gm. equivs. per liter; [Na] = 0.068; [Cl] = 0.630 (compare table 4). Usually, however, in those habitats where the growth of *V. macrophysa* was scanty and the plant deviated from its typical form it tended rather toward more irregular branching than the form characteristic of *V. ventricosa.* These facts are in accord with the generally accepted view that two distinct species are involved, but there still remains the highly suggestive fact that where either occurs abundantly the other is entirely lacking and that with changing conditions, sites (inner sea walls IV and V) previously conducive to the growth of one form (*V. macrophysa*) are now being freely colonized by the other (*V. ventricosa*). Transplant experiments (85) have shown that other plants, particularly members of the plastic genus *Caulerpa,* which occur at Tortugas either only on the reef or exclusively in the moat and were regarded hitherto as distinct species, are in fact ecological forms. The striking complementary distribution of *V. macrophysa* and *V. ventricosa* in the moat might give support to this possibility with reference to these plants and would be consistent with Doyle's (25) observations that the cytoplasmic inclusions of the two forms are indistinguishable. The long time necessary for successful transplants in the case of *Valonia* complicates this method of approach, and until such evidence has been acquired the possible identity of the two species should not be completely excluded. The range of sap composition (see tables 4 and 5) which the two forms tolerate has some interest in this connection.

Whether the two forms remain as valid species or are subsequently proved to be ecological forms, the factors which are responsible for their distribution are of considerable physiological interest; the more so since, as the two forms are characterized by quite different sap composition, they may shed light also upon this problem.

In the tidal zone the daily period during which algæ are submerged is a familiar factor which determines distribution (28). This factor can safely be eliminated in the present case. *V. ventricosa* and *V. macrophysa* can be collected from situations identical from this point of view. In the Mediterranean *V. macrophysa* is obtained by dredging; at Bermuda, according to

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[1] Branching was predominantly at the base, instead of irregularly as would be expected from this location, with the production of unusually long but regular vesicles, not unlike fig. 10 of Kuckuck (49).

Crozier (23), it occurs several feet below low tide level though in sheltered mangrove creeks. Crozier also states that it occurs though less abundantly "among other sea weeds on the reef." These facts are interesting as they indicate possible points of difference between the Bermuda and Tortugas forms.

According to Britton (9), at Bermuda *V. macrophysa* occurs "in sheltered places, as in mangrove swamps . . . attached to submerged rocks and logs," and again in the Bahamas (10) its habitat is described as "under shelving rocks at low water mark and on and among other algæ and corals in shallow water."

The following are possible determining factors in the relative distribution of *V. macrophysa* and *V. ventricosa*:

1. *Exposure to surf.* This, in addition to the purely mechanical effects which it implies, also maintains a narrower range of variation of temperature, dissolved oxygen, etc., than would commonly prevail in a confined body of water.

2. *Physical character of the surface.* The chemical nature seems unimportant. *Valonia* grows indiscriminately on masonry, coral, or tunicates. The physical character, its inclination or, if a vertical plane surface, whether there are eroded cavities, seems more important. It must be recalled that the factor of inclination also involves light intensity, which is much greater on a horizontal than an inclined surface.

3. *Composition of the medium.* In the confined body of water heavily colonized with plants, greater fluctuations in dissolved oxygen, carbon dioxide content, acidity, and even salinity are possible than in the open sea.

4. *Illumination.* The proximity of the massive structure of the fort and its bastions and the confining wall of the moat determines the inclination and duration of the direct sunlight which the plants in the moat receive.

It is evident that these factors are interrelated, and to select a single predominant one presents difficulties. It is the aspects mentioned under 1 and 2 which appear to be outstanding, and these are most strikingly exemplified by the inner and outer surfaces of the sea wall III of the moat of Fort Jefferson. In the case of *Halicystis*, Hollenberg found that its habitat was determined by shelter from the heaviest wave action, but individual plants were found exposed to direct sun and heavy surf. At Naples, where *V. macrophysa* occurs without apparently the protection afforded by the moat at Tortugas or the creeks of Bermuda, it is totally submerged, is obtained only by dredging, and is therefore protected by depth from the direct action of waves. In only two sites was *V. ventricosa* found at a considerable distance from a free inlet from the sea (fort walls II and VI, see fig. 1), and here the character of the surface may be regarded as more congenial to *V. ventricosa* than to *V. macrophysa*. Beyond the evident fact that *V. ventricosa*, owing to the currents to which it is exposed, is not subject to the wide fluctuations in its environment which *V. macrophysa* sustains, it is difficult to believe that the action of the surf has any other causal character than a mechanical one, though the physiological explanation of this remains obscure. In the moat there is a marked diurnal variation in oxygen content. The abundance of green plants produces supersaturation relative to oxygen during the day, and this falls at night. The maximum day level is higher and the minimum night level is lower than that which prevails at the seaward edge of the open reef, where, by measurement, the sea water is maintained by the surf in equilibrium with air. Table 2 gives dissolved oxygen data obtained in 1933 at the time the saps were collected for the diurnal series (table 7) from the moat pool on side IV and the moat on side III. There is, therefore, a wide diurnal fluctuation in dissolved oxygen content and a slight one in acidity which does not occur, except perhaps in small isolated tide pools, on the reef. Taylor (86) gave some salinity and temperature data for the moat, but in this investigation the moat did not differ significantly with respect to these factors from areas outside. The presence of *V. ventricosa* on the fort walls II and VI warns against a too sanguine acceptance of the attractive idea that the wide diurnal oxygen fluctuation in this isolated body of water represents the chief causal factor which determines the predominant *Valonia* species. However, since oxygen data from these two sites are wanting, the possibility remains that in the absence of the rich growth of *Halimeda*, *Acetabullaria*, *Penicillus*, *Udotea*, etc., so conspicuous a feature of the vicinity of the ledge on side III, these localities were not subject to so wide a fluctuation.

TABLE 2—*Diurnal fluctuation in oxygen content and* pH *in the moat of Fort Jefferson*

Site	Conditions	Dissolved O_2*	pH
Moat III, ledge level	Low tide: 9:00 a.m.	80.9	8.3
	6:00 p.m.	150.0	8.6
Moat pool IV	Low tide: 9:00 a.m.	48.7	8.3
	6:00 p.m.	184.0	8.7

* Sea water saturated with air=100.

In short, the difference in oxygen relations which the two types of habitat entail seems to be the only alternative to purely mechanical effects as an explanation of the pronounced effect of surf upon the distribution of the two species.

Light does influence *V. macrophysa* in ways to be described later, but this organism can and does exist over such a range of light conditions that it is improbable that this factor is responsible for the complementary distribution of *V. macrophysa* and *V. ventricosa* at Tortugas. Relative to light, the outer sea wall III (*V. ventricosa*) is not unlike the inner sea wall VI (*V. macrophysa*), while the fort wall VI, where *V. ventricosa* occurs, is not greatly different in its orientation to light from the inner sea wall III (*V. macrophysa*). The inner and outer sea wall III both receive full direct sun for a considerable part of the day, the only difference being that in the one case (inner surface) this occurs just before noon and in the other (outer surface) shortly after noon. Of particular interest is the fact that Taylor in 1924–1926 (86, p. 26) definitely reported *V. macrophysa* from the inner sea wall on side IV, in areas in which the only *Valonia* present in 1933, though the light conditions remain unaltered, was *V. ventricosa*. It seems, therefore, that light cannot be the paramount factor in question.

A further general point deserves mention. The age and longevity of these large multinucleate vesicles seems to be unknown. Whether they grow entirely by protracted, slow expansion or have a period of rapid expansion and synthesis followed by a period of slow growth during which the wall thickens is still not known. A recent study (40, 41) of *Halicystis ovalis* has shown that in this plant the large vesicles are the growth of one year. The vesicles develop in the summer later than March, disappear in the fall, and are produced successively by the same plant, which has a definite "rhizome" which penetrates the substratum. There seems to be no method whereby the age of the largest individuals of *V. ventricosa* can be precisely estimated from available records. Since the collections from the dock of Loggerhead Key in 1934 had probably appeared since 1933, this suggests that vesicles at least 10 cc. in volume can grow in one year. The assumption that *V. ventricosa* appeared in the moat since Taylor's survey places an upper limit of 10 years upon the age of the largest vesicles, namely 24 cc., found on Garden Key, but it is doubtful whether this estimate has any great value. Conversely, the fact that certain stations which were stripped of large vesicles in 1934 had not regained them by 1937 suggests that several years are necessary for the growth of the largest specimens. The observation that large plants are more abundant in those areas of the seaward edge of Bird Key Reef which become accessible only at the lowest tides, and are not therefore so frequently collected, suggests that in a normal habitat the vesicles may survive from one year to another. The greater frequency of large specimens on newly collected sites (outer sea wall III and IV and the fort wall of the moat and an area on Bush Key observed in 1937) points in the same direction. The fact that the large vesicles can be kept in aquaria for months is now familiar and is indicative of their longevity. The largest perfect individuals of *V. ventricosa* which were encountered at Tortugas during 1933 and 1934 had volumes of 30 cc. and 24 cc. respectively, though even larger ones were found in 1937 (see Plate 2). The relative frequency of the vesicles according to size may be derived from the curve shown for collections made during a period of low tides when rich collections were secured from Bird Key Reef (fig. 2). Vesicles smaller than those of class 6, table 3, were not collected. It is an obvious supposition that size is a measure of age. The only direct evidence in support of this is that the smaller vesicles are usually most free from calcareous incrustation and the large ones not only are more incrusted but often support other algæ growing as epiphytes.

In 1937 an area not collected intensively in recent years was found to be unusually rich in *Valonia ventricosa*. This area yielded more vesicles per unit area than any other known station at Tortugas, and the proportion of large vesicles was unusually large (6.7 per cent of a population of 800 having a diameter greater than 2.20 cm.).

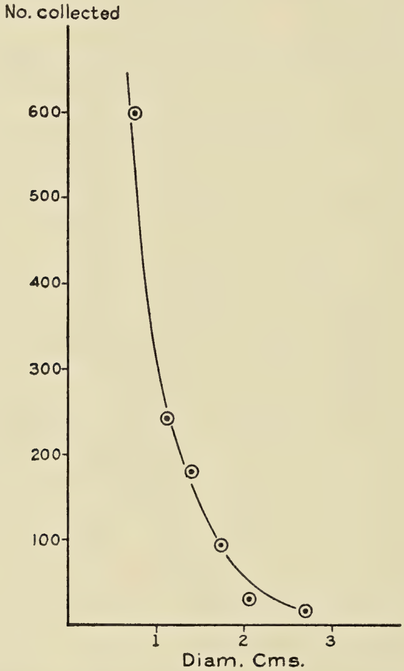

Fig. 2—Relative frequency of vesicles according to size, *Valonia ventricosa.*

As far as *V. macrophysa* is concerned, the quantity of material for experimental purposes presents no problem. This plant may be removed in dense tufts from the masonry on which it grows, and from these a large population of individuals may be obtained. It is evident, however, that every collection must include vesicles of all ages, and for this plant, in which the vesicles are of the first, second, or even third order (49), the size of single vesicles has even less significance as an index of their age. It is frequently stated that at Bermuda the vesicle attains the size of a "pigeon's egg" (e.g. 61, p. 130). Unusually large examples to which this description might apply occur at Tortugas, but the vesicles do not often exceed a volume of 1 to 2 cc.

During his visits to the Fort, Taylor noted qualitatively that the *V. macrophysa* became more abundant as the season progressed, and this implies that it was still actively growing in June and July (see 86, p. 25). Collected specimens of the Bermuda form are reputed to grow only in the winter (62). Only sluggish expansion of collected vesicles of *V. ventricosa* occurs under laboratory conditions, but Kopac (47) observed relatively rapid growth of the sporelings which were produced in response to mechanical stimulation. In the past few years repeated references have been made to the growth of *V. macrophysa* during laboratory experiments. That the growth rate may attain 1 per cent per day is claimed by Osterhout. M. M. Brooks (12) reported linear measurements on dissected vesicles of *V. macrophysa* during 68 days at 22° C. The increments (6 per cent in length, 22 per cent in width) were not large. The change of shape was ascribed to the removal of the compression of surrounding "cells." Of still more interest is the observation of Doyle (25) that on freshly segregated vesicles rhizoidal cells develop from initials the development of which was prevented in the large mass. It is possible that this, which must surely occur during the so-called "seasoning process" to which *Valonia* is commonly subjected prior to its use in experiments, explains much of the efficacy of this treatment and may be the most significant growth of which the vesicle is capable. The more recent experiments from Bermuda which show signs of extension are almost always concerned with small vesicles, for example not exceeding 0.5 cc. (44), and even so the total increments of volume during experiments are not very impressive (e.g., 2 ml. on 11.40 ml.; see *loc. cit.*, table 2). The most important point is, however, that mere extension hardly justifies the conclusion that the organism is growing. The production of new substance, division of nuclei, even branching would more nearly express the properties of constructive metabolism which now seem to be necessary for active salt accumulation (80, 81, 69). In the writer's view, until *V. macrophysa* can be cultured, its "growth" in the form of isolated vesicles must be regarded as mere sluggish extension and not necessarily comparable

TABLE 3—*Frequency of occurrence of Valonia ventricosa according to size of vesicle* *

Class	Diameter limits (cm.)	Mean diameter (cm.)	No. collected	Relative frequency
1.......	3.20–2.20	2.70	22	1.0
2.......	2.20–1.90	2.15	32	1.4
3.......	1.90–1.60	1.75	95	4.3
4.......	1.60–1.25	1.42	180	8.2
5.......	1.25–1.10	1.18	241	11.0
6.......	1.10–0.50	0.80	600	27.0

* Since it has been a common practice, also adopted to some extent in this work, to analyze a composite batch of sap derived from vesicles not specially controlled as to size, it is noteworthy that the data of table 3 imply that the bulk of the sap from random samples would be derived from the vesicles of medium size, i.e. 2.00 to 1.25 cm. diameter.

to its normal growth in situ, which implies in addition increase of "cells" and substance (see also 80).

SUMMARY OF SECTION I

Valonia macrophysa occurs at Tortugas only in the moat of old Fort Jefferson. This organism demands complete protection from the effects of swell and surf. The growth obtained on a horizontal ledge is luxuriant; that on an inclined or vertical surface sparse and irregular. In the protected locations it demands, *V. macrophysa* is exposed to, and withstands, a wide range of light conditions and diurnal fluctuation in the composition of the external medium (oxygen and carbon dioxide content and acidity).

Valonia ventricosa is abundantly obtained on Bird Key Reef. It has been collected, however, from other stations both within and without the moat. Its distribution is determined by the converse of the factors which apply to *V. macrophysa.* It requires but slight protection from swell and surf. It tolerates a vertical substratum and grows freely attached to minute crevices in masonry or coquina rock, but is intolerant of an environment subject to diurnal fluctuation.

The distribution of *V. ventricosa* is complementary to that of *V. macrophysa,* and the solution of the problem whether the species are distinct, raised thereby, must await adequate transplant experiments. However, specimens of the two species growing in close proximity in the moat retained their salient characteristics of form and sap composition.

The size of the vesicles of *V. ventricosa* is discussed with reference to the problem of their age and frequency of occurrence. The physiological importance of certain cytological and other features of the vesicle, which have not received the attention they deserve, is emphasized.

II. THE SAP COMPOSITION OF VALONIA MACROPHYSA AND V. VENTRICOSA IN CONTACT WITH SEA WATER

Mainly during 1934 but to some extent in 1933 a large number of sap samples were obtained from both species of *Valonia* growing in the various stations already described.

Composite samples were obtained from *V. macrophysa* without dissecting the palisade-like masses in which the alga was removed from the substratum. The vesicle to be punctured was surface-dried with filter paper, pierced with a fine pipette having an attached rubber teat, and the sap then extracted by gentle suction. A quantity of sap sufficient for analysis was accumulated at each collection. Unless otherwise stated, the sap was obtained when the plants were collected and without bringing them to the laboratory.

The treatment of *V. ventricosa* was similar. Though some saps were extracted without even removing the plants from their substratum, this procedure was far too cumbersome for general use, and the data available suggest that no appreciable errors were incurred by the simpler procedure. The sap was extracted either during the return journey in the launch to the laboratory or immediately upon arrival there. In the interval between collection and sap extraction, a period rarely much in excess of an hour, the plants remained in sea water and were protected alike from bright light and from high temperatures. In the case of *V. ventricosa* the sap extraction was best effected by the use of a long, fine glass needle drawn from a rod and tapered to a sharp point. The sap emerged from the transfixed vesicle when the needle was slightly withdrawn, and the protruding tip of the needle served to direct the slow stream of sap into a clean, dry vial. The specimens of *V. ventricosa* were usually graded according to size. Both composite sap samples and sap from single plants have been analyzed.

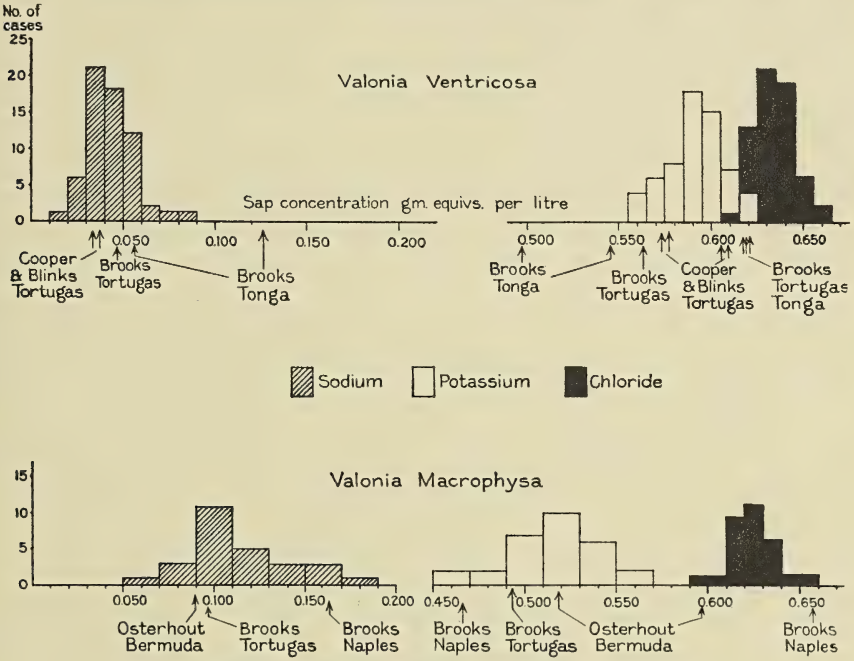

FIG. 3—Range of sap composition of *Valonia* at Tortugas.

A full description of the analytical methods used for the determination of potassium, sodium, and chloride and a discussion of the errors and difficulties encountered will be found in the Appendix to this paper.

In addition to the samples so obtained that the sap composition reflects that of the plants in their habitat, other data accrue from the sea-water controls in various laboratory experiments. These sap samples were from vesicles which had been collected, superficially cleaned, graded, and kept under clean conditions in the diffuse light[1] of the laboratory either in an aquarium with a slow stream of sea water or in large dishes in which the sea water was renewed daily.

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[1] Estimated intensity relative to the vertical component of full sunlight, from photoelectric measurements, of the order of 0.1 to 0.5 per cent.

In all, sixty-two complete analyses are available for *V. ventricosa* and thirty for *V. macrophysa.* Upon the basis of these figures may be assessed the range of sap composition which these organisms exhibit when they are in contact with normal sea water under the influence of the variable environmental conditions provided by their habitat and the laboratory.

Figure 3 summarizes the data in the form of frequency curves. The units for grouping were arbitrarily selected and may be ascertained from the curves. The complete data for each population are not assembled in separate tables, although many of them occur in other parts of this paper.

It is immediately evident that the sap composition is not constant. For each species and each solute (K, Na, Cl) the range encountered is far greater than the errors of analysis. The conception that the sap composition of these species can be precisely described upon the basis of isolated collections is without foundation.

The two species may be characterized and compared by determining for each constituent the mean, the standard deviation, and the standard deviation of the mean from the data available, which are apparently almost normally distributed. These data are given in table 4 and were calculated according to methods described by Fisher (26). For each constituent (K, Na, or Cl) the mean value in column 3 necessarily is more representative than the scanty figures which have sufficed hitherto. Nor must it be supposed that the mere collection of a large composite sap sample is an adequate substitute for the larger number of collections here reported. In all but a very few of the cases here involved, the sap sample was a pooled one from many vesicles. If all the saps analyzed had been pooled into one composite sample, even if the same mean had been obtained thereby, no

TABLE 4—*Composition of the sap of Valonia ventricosa and V. macrophysa at Tortugas*
(Sap concentrations in gm. equivs. per liter)

Species	Ion	1 Mean	2 Mean ± 2 × S.D.	3 Mean ± 2 × S.D. of mean
V. macrophysa (No. of samples 30)	K	0.509*	0.569–0.449*	0.520–0.498*
	Na	0.113	0.167–0.059	0.123–0.103
	Cl	0.624	0.647–0.601	0.628–0.620
V. ventricosa (No. of samples 62)	K	0.591	0.621–0.561	0.595–0.587
	Na	0.043	0.067–0.019	0.046–0.040
	Cl	0.628	0.650–0.606	0.631–0.625

* Determined as cobaltinitrite but corrected to the chloroplatinate basis. See Appendix, p. 166.

Column 2 for use when considering single determinations.

Column 3 for use when considering means.

estimate of the range and variability would have been possible. Column 2, however, gives for each constituent (K, Na, Cl) the reasonable limits within which single sap determinations would be expected to lie if they were withdrawn from populations not significantly different from that represented by the samples analyzed. These limits may justifiably represent those characteristics of the sap of the *Valonia* species in question at Tortugas, at least until even more extensive data become available. Column 3 gives the limits within which the mean value of several determinations of sap concentration could lie without necessarily indicating that the samples from which the sap was obtained differed significantly from the population from which these results were recorded.

Clearly only mean values with their errors assessed form an adequate basis for comparison of the sap of different *Valonia* species or for an estimate of the effect of experimental treatment or of habitat factors upon the sap composition of the same species. Brooks (17) urges that data upon the sap composition of marine algæ may have a taxonomic value. Any such applications could be made only after the range and variability in sap composition had been adequately estimated. With reference to the acidity of the sap, Crozier estimated the pH range (5.0 to 6.7) obtained from 50 single "cells" at Bermuda and calculated the mode. In terms of single vesicles the sap would no doubt show even greater variance than that indicated by table 4.

It is evident, both from figure 3 and from table 4, that the previous records of the sap composition of *V. macrophysa,* whether obtained from Naples, Bermuda, or Tortugas, are all included within the scope of this distribution obtained at Tortugas. The conclusion cannot be avoided that the previously published figures are not typical of the organism in question. Single arbitrarily selected samples cannot represent the sap composition of an organism which displays a much greater range and variability than previous writings imply. One set of analyses by Cooper and Blinks (21) was published only in relative and not absolute units and cannot, therefore, be compared with the above. With respect to *V. macrophysa* it is evident that many samples were obtained from Tortugas which had potassium content considerably higher, not only than the two previously recorded figures (Blinks and Brooks) from Tortugas, but also than the "standard" Bermuda analysis. It happens that the mean of the thirty-five determinations falls very close to the single Bermuda figure; therefore, approximately half of these exceeded it, some by considerable amounts. A later section will make it evident that these saps, richest in potassium, were obtained directly from plants growing in the moat of Fort Jefferson. Emphasis is placed upon this fact because it shows that the argument that, because of its allegedly low content of potassium, the *V. macrophysa* at Tortugas is not "healthy in the summertime" and is "contaminated with the ions of sea water" is groundless. On the contrary, during this survey the highest potassium and the lowest sodium concentrations yet recorded for *V. macrophysa* were encountered, and these in samples obtained in the "summertime" from the moat of Fort Jefferson. This erroneous impression arises from the comparison of a single and clearly unrepresentative analysis from Tortugas with a single and old analysis from Bermuda, which, if the necessary data were available, might not prove to be any more typical even of that locality than is that made by Cooper and Blinks for Tortugas. Until many more analyses become available from these stations it is futile to speculate whether there is any real difference between the sap content of the *Valonia* growing at Bermuda and that at Tortugas. The existing figures suggest that the differences in sap composition encountered within the Tortugas area itself are as great as, or greater than, those which are to be anticipated from even wide differences in geographical location. Since the records permit some further analysis of the causes of the variability encountered at Tortugas, this will be attempted in a later section.

In the case of *V. ventricosa,* comparison can only be made with other data from Tortugas (Brooks [17] and Cooper and Blinks [21]), and from Tonga in the Pacific (Brooks). Valid *V. ventricosa* apparently does not occur at Bermuda. Only the Tonga data lie outside the range of sap composition obtained at Tortugas by an amount which represents a real difference. This applies only to potassium (which is low) and sodium (which is high), but not to chloride, which falls within the limits obtained at Tortugas. The previous records of Brooks and of Cooper and Blinks, the latter for "cells freshly collected" and "after three weeks in a closed bottle of sea water," all fall within the limits typical of this species at Tortugas.

The balance of the principal cations (K and Na) against the principal anion (Cl) is of some interest. It has been a common practice to compare the composition of these saps relative to the halide concentrations as 100. Where this obscures absolute variations the practice is to be deprecated. The figures already given for the mean sap composition are of interest in this connection (see table 5). Clearly the combined potassium and sodium balances the chloride quantitatively. The same conclusion may be verified by inspection of the analyses of individual sap samples, where the discrepancies rarely exceed the legitimate and small errors of these determina-

TABLE 5—*The mean sap composition of Valonia*
(Sap concentrations in gm. equivs. per liter)

Species	Mean [K]	Mean [Na]	[K]+[Na]	Mean [Cl]
V. ventricosa........	0.591	0.043	0.634	0.628
V. macrophysa.......	0.509	0.113	0.622	0.624

tions. Sulphate was invariably absent from the saps. Calcium and magnesium were not determined on most of the samples. The records given in table 6, however, show that, as is generally believed, the concentration of these constituents is small in comparison with that of potassium and sodium.

TABLE 6—*Calcium and magnesium in the sap of Valonia*
(Sap concentrations in gm. equivs. per liter)

Species	Site of collection	Ca	Mg
V. macrophysa......	Moat side III, 1933	0.0050	0.0090
		0.0054	0.0079
		0.0054	0.0047
V. ventricosa.......	Bird Key Reef, subsequently in laboratory aquarium	0.0020	0.0120
		0.0020	0.0090

This is an appropriate place to record that Mr. Ulrich, of the Division of Plant Nutrition, University of California, has examined the sap of *V. ventricosa* for organic anions of non-volatile, ether-soluble acids. The value obtained (0.0015 equivs. per liter of sap) seems too low to have any bearing upon the balance of anions and cations in the sap. These results, therefore, in agreement with those of Cooper and Blinks, again demonstrate that the sap of both *Valonia* species at Tortugas may be considered for all practical purposes as a mixed solution of the chlorides of potassium and sodium.

The comparison of the two species is of some interest. It has often been emphasized (58, 59, 21, 17) that *V. macrophysa* is poorer in potassium and richer in sodium than *V. ventricosa*. This difference is so big that it is not vitiated by the scanty number of samples upon which it is usually based. Figure 3 and table 4 reveal the same result. The difference between the mean potassium concentrations is 0.082 gram equivalents per liter, and since this difference has a standard error of only 0.006, its significance cannot be doubted. In the same way the higher sodium concentration of *V. macrophysa* could not possibly be due to chance. The difference between the means is 0.070 and its standard error is only 0.005. The comparison with respect to chloride yields a different result. The difference between the respective means (0.004) is small with respect to twice its standard error (0.012). Therefore, by their chloride content these two species as they occur at Tortugas are indistinguishable; a fact which is also made evident by visual comparison in figure 3. In the event that the two organisms proved to be growth forms, rather than distinct species, this fact would be important.

SUMMARY OF SECTION II

The range of sap composition which *V. ventricosa* and *V. macrophysa* exhibit at Tortugas in sea water is described. The sap composition is subject to greater variation than existing tables which purport to give the composition of the sap imply.

The limits for the two species at Tortugas have been set by calculating the mean, standard deviation, and standard deviation of the mean from the figures available.

Sap analyses previously recorded for these species, whether from Tortugas or from other localities, fall within the range described.

Comparisons between species and between specimens of the sap of the same species in different localities should be made only upon the basis of the mean sap composition with an estimate of the degree of variability. Attention is drawn to certain fallacies which have arisen because these considerations have not been appreciated.

The combined potassium and sodium concentration is balanced by the chloride concentration. Data confirm previous results which show that the concentrations of calcium and magnesium are small.

Comparisons made between the mean sap composition of *V. ventricosa* and of *V. macrophysa* confirm the conclusion that the former has the greater potassium and lower sodium concentration, the differences being significant; but there is no significant difference of chloride concentration.

III. VARIABLES WHICH AFFECT THE SAP COMPOSITION OF VALONIA IN NORMAL SEA WATER

The preceding section deals with the range of sap composition which *V. ventricosa* and *V. macrophysa* exhibit in contact with sea water, unmodified as to its composition, at Tortugas. It now remains to specify some of the factors which, within these limits, determine the sap composition.

VALONIA MACROPHYSA

A striking difference in the sap composition of *V. macrophysa* from different parts of the moat was observed in 1933. During a fruitless attempt to ascertain if there was a diurnal variation[1] in the sap, collections were made three times during the same day from each of two selected stations. One of these was the ledge level at the northern end of side III (fig. 1) and the other the tide-filled pool, isolated from the main body of the moat by a broad, high bank of coral fragments, which was situated in the shelter of the northeast bastion of wall IV and is referred to on figure 1 as the moat pool of 1933. These sap analyses are recorded in table 7.

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[1] Crozier (23) described the diurnal variation of the acidity of the external medium containing *Valonia* as early as 1919, but found the internal pH constant.

The outstanding fact which emerged in 1933 was the very low potassium and high sodium content of *V. macrophysa* from the moat pool. As described earlier, this pool of 1933 disappeared before 1934, but an almost identical analysis was obtained from plants growing in a similar isolated pool in an adjacent site to that of 1933 (see fig. 1). Several analyses from wall III in 1934 reproduced closely the figures of 1933 (a fact which is of some interest with respect to seasonal variations), from which it is concluded that this difference between moat and moat pool is a real one determined by some factor of these adjacent habitats.

The wider diurnal fluctuation in the dissolved oxygen content in the moat pool than in the main body of water on side III has been mentioned (p. 104). This may be a contributory factor, but the outstanding difference between these two positions seems to concern light. The moat pool of 1933, shaded by the high fort and its bastions, received direct sunlight for only a short period in the afternoon. Furthermore, the *Valonia* was shaded from even this direct light by the bank of coral fragments. In contrast, the moat ledge side III received direct sun from about 9:00 a.m. until just after noon, except at its southern end, where a shadow thrown by the bastion shortened this period considerably. With a suitably mounted[1] Weston Photronic Cell attached to a microammeter, with shunts to register the fractions 1.00, 0.10, 0.01 of the total current, measurements of the total light intensity were made. Factors were available for the conversion of microamperes to candlepower at each shunt value. The figures given in table 8 compare the two sites. The column headed "Microamperes×shunt" represents an

TABLE 7—*Diurnal variation in sap composition of Valonia macrophysa*
(Sap concentrations in gm. equivs. per liter)

Site	Date	Time	[K]	[Na]	[Cl]
Moat, ledge level, wall III	Aug. 16, 1933	9:00 a.m......	0.531	0.102	0.633
		3:00 p.m......	0.534	0.115	0.652
		7:00 p.m......	0.527	0.106	0.633
Moat pool, wall IV	Aug. 16, 1933	9:30 a.m......	0.453	0.164	0.623
		4:00 p.m......	0.471	0.155	0.644
		7:00 p.m......	0.427	0.180	0.622
Moat, ledge level, wall III	July 30, 1934	9:30 a.m......	0.537	0.090	0.630
		11:00 a.m......	0.537	0.091	0.630
		4:00 p.m......	0.527	0.095	0.625
		7:30 p.m......	0.551	0.075	0.625
Moat pool, wall IV	July 21, 1934	11:00 a.m......	0.451	0.150	0.592

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[1] We are indebted to Mr. P. R. Stout, of the Division of Plant Nutrition, University of California, for help with the construction of this apparatus.

approximate measure of the vertical component of the light intensity at the water surface.

The obvious suggestion is that the low potassium content of *V. macrophysa* from the moat pools was determined by the short duration of high light intensity to which it was subjected. This prompted the analysis of the available results which is represented by table 9. In this classification all collections made from sites which received for long periods the high light intensities of the direct sunlight of late morning or early afternoon are placed in group I, designated the "high light" group. Twelve independent collections of this kind are available, and despite considerable disparity in the times of their collection and analysis they represent a strikingly homogeneous group. Collections from sites[1] which clearly suffered from greater shading due to the shadows of the fort or its bastions, or which received only the oblique illumination of early forenoon, appear in group II, which is designated the "medium light" group. The afore-mentioned samples from side IV, obtained from the extreme shade, appear in group III, which is designated the "low light" group. Group IV includes data which apply to material collected, carefully dissected to single vesicles, and allowed to adjust to sea water and diffuse light. The latter group does not enter into this first comparison, which concerns only the sap composition of plants in situ.

Table 8—*Light intensity at the moat pools on side IV and the ledge of the moat, side III*

Site	Date	Time	Microamperes	Shunt	Microamperes × shunt	Total flux (foot-candles)
Moat wall III, water surface above ledge	July 16	9:00 a.m......	44	110	4,840	6,100
		10:00 a.m......	60	110	6,600	8,600
		11:30 a.m......	76	110	8,360	12,000
		2:00 p.m......	26	10	260	290
		3:00 p.m......	18	10	180	200
Moat wall III, water surface above ledge	July 21	9:30 a.m......	53	110	5,830	7,400
Moat pool	July 21	9:00 a.m......	15	10	150	170
Moat wall III, water surface above ledge	July 24	2:30 p.m......	22	10	220	250
Moat pool	July 24	2:45 p.m......	67	100	6,700	8,700
		4:00 p.m......	reading not taken but pool in shadow.			

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[1] Photoelectric readings were obtained in the different parts of the moat, but these are omitted.

TABLE 9—*The effect of habitat on the composition of the sap of Valonia macrophysa*
(Sap concentrations in gm. equivs. per liter)

I "High light" group				II "Medium light" group				III "Low light" group				IV Collected vesicles in diffuse light of laboratory		
Site	[K]	[Na]	[Cl]	Site	[K]	[Na]	[Cl]	Site	[K]	[Na]	[Cl]	[K]	[Na]	[Cl]
								Moat pools of side IV	0.451	0.150	0.592	0.510	0.099	0.623
									0.453	0.164	0.623	0.517	0.099	0.625
Ledge on moat wall III, out of shadow of south bastion.	0.537	0.090	0.630	Ledge on moat wall II, shaded by wall and S.E. bastion	0.515	0.105	0.615		0.471	0.155	0.644	0.495	0.129	0.625
	0.537	0.091	0.630						0.427	0.180	0.627	0.495	0.129	0.625
	0.527	0.095	0.625									0.495	0.118	0.625
	0.551	0.075	0.625									0.481	0.133	0.608
	0.531	0.102	0.633	Ledge on moat wall III shaded by S. bastion	0.512	0.102	0.615					0.494	0.127	0.623
	0.534	0.115	0.652		0.509	0.102	0.615					0.491	0.139	0.617
	0.527	0.106	0.633									0.498	0.140	0.625
				Fort wall VI......	0.513	0.102	0.619							
Fort wall III..	0.520	0.096	0.619											
	0.532	0.078	0.619											
Fort wall II...	0.524	0.080	0.615											
Ledge on moat wall V	0.530	0.094	0.630											
Ledge on moat wall VI......	0.520	0.095	0.619											
Mean	0.531	0.093	0.628	Mean	0.512	0.103	0.616	Mean	0.450	0.162	0.621	0.497	0.123	0.622
S.E. of mean.	0.0027	0.0034	0.0033	S.E. of mean....	.....	.....	.....	S.E. of mean....	.....	.....	.....	0.0011	0.0052	0.0020

The significance of the differences in the mean sap composition for groups I, II, and III may be tested by the "t" test (Fisher, 26, p. 120). Comparing groups I and II relative to potassium, $n=14$ and $t=3.93$, so that the value of P, <0.01, leaves no doubt as to the reality of the difference between them. The difference between groups I and III has even greater significance, while the comparison of groups II and III ($n=6$, $t=6.75$, and $P<0.01$) again indicates that the difference could not be due to chance variations in the samples. Similarly the comparison of the mean sodium concentration of groups I and II leads to the values $t=2.61$, $n=14$, and $P=0.02$, so that the difference may be regarded as significant. The comparison of groups I with III and II with III on the basis of the sodium content yields high values of t and low values of P, which place a high degree of certainty upon the reality of the differences in question. The differences of potassium and sodium content in groups I, II, and III are, therefore, real, and the conclusion is that light was the causal factor involved. The highest potassium and lowest sodium concentration were produced by the maximum illumination during growth. This appears to be the first record that light influences the *concentration* of the salts normally in the sap of *V. macrophysa.* The effects of light referred to by Osterhout (44) were only upon the growth rate.

It remains to consider group IV. The factors which operate during growth and which determine the sap composition are no longer in question, but rather those changes which are superimposed and incidental to the technique of collecting, dissecting, and adjusting the plants in glass containers to renewed sea water and diffuse light. All the plants in question came originally from sites represented by group I. It is quite evident that relative to group I, group IV has lower potassium and higher sodium content. The "t" test indicates that the difference relative to potassium content ($t=10.4$, $n=19$, P very small) is highly significant, and for sodium ($t=4.77$, $n=19$, $P<0.01$) a similar conclusion is indicated. Although the difference in the mean chloride concentration for the two groups does not attain the level of statistical significance (being only 1.58 times its standard error), it is still of some interest that its direction is the same as that observed for potassium, indicating that the effect in question concerns not merely the relative proportion of potassium and sodium, but also the total concentration of salt.

There is, then, the interesting conclusion that collected plants coarsely dissected to small numbers of vesicles and allowed to adjust to diffuse light and sea water show a decline in potassium (and possibly also in chloride) and a gain in sodium relative to the values which obtained when they were still attached in their habitat. No doubt this in part explains the fact that the figures previously reported for the sap composition of *V. macrophysa* have lower potassium and higher sodium concentrations than the mean of the distributions given in this paper. However, of greater interest from the point of view of this analysis is the fact that the new salt level to which the plants adjust after collection is one which would be typical of a poorer light supply during growth, and this is not inconsistent with the actual light conditions to which they were exposed in the laboratory. It is also suggestive that a great many of the plants used experimentally at Bermuda had sap poorer in potassium than the standard analysis which is the one which seems most likely to represent their composition in situ in this locality.

In laboratory experiments which involve *V. macrophysa,* the plant is rarely used in the dense masses in which it is collected. The vesicles are usually separated one from another and at the same time freed from associated epiphytes and roughly graded according to size. The effect of the inevitable manipulation involved upon the composition of the sap must be considered.

The immediate effect may be great. Even when the separated vesicles show no visible signs of injury the potassium concentration may fall as low as 0.396 M., though the chloride concentration remains approximately constant. This is mainly due to exit of potassium and entry of sodium, but may be in part due to simultaneous loss of potassium and chloride and in part due to water uptake caused by the release of pressures from adjacent vesicles. The detailed mechanism involved is unknown. Presumably it has its basis in mechanical disturbance to the protoplast. The symptoms induced by controlled experimental injuries, both reversible and irreversible in character, have been described by Kopac (47, 48), and also the attendant effects upon the electrical properties of the protoplasm. Similar effects may occur during the coarse dissection of separate vesicles, although at this juncture nothing is to be gained by attempting to attach causal significance to the resultant electrical phenomena with regard to the loss of potassium which ensues. Subsequently, however, if the dissected material remains undisturbed under clean conditions in sea water the potassium and chloride content again increases and readjusts to the level typical of *V. macrophysa* in sea water in the diffuse light of the laboratory (table 9). During this process potassium is accumulated, largely by exchange for sodium, and in the early stages of recovery the quantities involved may be considerable. It seems necessary, however, to discriminate between this accumulation of potassium which occurs in response to an induced subnormal concentration in the sap from that primary uptake of potassium during growth when no such condition prevailed.[1] There can be little doubt that the disputed (43, p. 545) uptake of potassium which Brooks claimed (13) to observe even in modified sea water of low potassium concentration, and which he cited as evidence of a non-equilibrium condition, can be attributed to these causes (78). The workers at Bermuda habitually submit the collected plants of

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[1] For a discussion of the considerations involved here see Steward (80).

V. macrophysa to a so-called "seasoning process"[1] before they are used experimentally. Presumably this is analogous to the process described above. However, the actual salt level to which the sap concentration is adjusted thereby has implications with respect to the subsequent potentiality for salt accumulation under any other conditions which still remain obscure. It may be significant that the composition of the sap from plants so treated (42, tables 1 and 2) showed that, though somewhat higher in chloride,[2] they were actually poorer in potassium and richer in sodium than the figures which commonly represent the analysis of sap at Bermuda.

VALONIA VENTRICOSA

The data summarized by the distribution curve shown in figure 3 may be subdivided into the groups shown in table 10. In groups I to IV the samples were collected from the places specified and the sap extracted quickly by the procedure described on pages 108–109. In Group V large numbers of plants were collected mainly from Bird Key Reef, but also from the pylons on Loggerhead Key, and stored in the diffuse light of the laboratory (approximately 0.5 per cent of the vertical component of sunlight at noon) in glass dishes in which the sea water was changed periodically. The figures listed in group V represent random samples from this stock which, still remaining in sea water, acted as controls during various laboratory experiments. Table 10 shows the data for K, Na, and Cl respectively according to this grouping. Casual inspection of the mean figures suggests that there was no difference in the composition of the *V. ventricosa* samples of groups I and II. This impression may be readily confirmed by the "t" test, and in most subsequent comparisons the combined data from groups I and II will be treated as a single unit. The identity of these two stations is not unexpected. Both provide the minimum of mechanical protection, full exposure to the surf, and only a partial shading, which permits the plants to receive light of high intensity, at least during part of the day.

Groups III and IV provide the interesting comparison of the *V. ventricosa* growing on the vertical outer surface of the confining wall of the moat with that which occurred within the moat in the places already described (fig. 1).

The samples collected from plants growing on the seaward surface of the confining wall of the moat had a lower chloride concentration than those collected from *V. ventricosa* growing within the moat. The difference (0.013 gm. equivs. per liter) is a significant one since $t=3.18$, $n=16$, and the value of P is, therefore, much less than 0.01; so that the result would not have occurred by chance once in over a hundred similar trials. The similar comparison made for potassium and sodium yielded only differences which are

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[1] For a description of this procedure see Jacques and Osterhout (42, p. 302).

[2] Owing, no doubt, to their small size—smaller than the vesicles from a stand likely to be chosen for a large sap sample.

The effect of habitat on the composition of the sap of Valonia ventricosa
(Sap concentrations in gm. equivs. per liter)

TABLE 10

I Loggerhead Key, pylons of laboratory wharf			II Bird Key Reef, interstices of coral and coquina rock			III Seaward surface of moat walls III and IV of Fort Jefferson			IV Moat of Fort Jefferson (fig. 1)			V Plants from Loggerhead Key and Bird Key Reef adjusted to still, changed sea water in laboratory		
[K]	[Na]	[Cl]	[K]	[Na]	[Cl]	[K]	[Na]	[Cl]	[K]	[Na]	[Cl]	[K]	[Na]	[Cl]
0.574	0.039	0.611	0.557	0.061	0.615	0.570	0.051	0.611	0.587	0.042	0.623	0.602	0.046	0.654
0.562	0.053	0.611	0.570	0.050	0.617	0.593	0.034	0.627	0.615	0.042	0.642	0.591	0.051	0.639
0.575	0.045	0.619	0.595	0.021	0.617	0.610	0.035	0.637	0.621	0.030	0.642	0.577	0.056	0.631
0.567	0.054	0.615	0.599	0.050	0.639	0.595	0.022	0.615	0.605	0.034	0.637	0.616	0.043	0.654
0.582	0.039	0.625	0.557	0.071	0.609	0.596	0.017	0.615	0.593	0.055	0.644	0.604	0.042	0.639
0.583	0.031	0.617	0.583	0.037	0.611	0.607	0.035	0.623	0.621	0.029	0.644	0.609	0.035	0.639
0.594	0.028	0.625	0.584	0.041	0.620	0.599	0.038	0.625	0.611	0.021	0.644	0.609	0.041	0.635
			0.592	0.041	0.624	0.602	0.037	0.623	0.610	0.024	0.630	0.595	0.035	0.630
			0.583	0.045	0.628				0.586	0.048	0.625	0.597	0.038	0.630
			0.588	0.051	0.632				0.595	0.031	0.625	0.599	0.052	0.636
												0.591	0.036	0.636
												0.599	0.033	0.639
												0.602	0.047	0.639
												0.590	0.045	0.639
												0.613	0.047	0.635
												0.595	0.053	0.639
0.577	0.041	0.617	0.581	0.047	0.621	0.597	0.034	0.622	0.604	0.036	0.635	0.599	0.044	0.638

TABLE 11

Ion	Exposed group (I and II)		Partially protected group (III)		Protected group (IV)		Collected plants (V)	
	Mean conc.	S.E. of mean	Mean conc.	S.E. of mean	Mean conc.	S.E. of mean	Mean conc.	S.E. of mean
K................	0.579	0.0032	0.597	0.0043	0.604	0.0042	0.599	0.0023
Na...............	0.044	0.0028	0.034	0.0036	0.036	0.0034	0.044	0.0018
Cl................	0.620	0.0020	0.622	0.0029	0.635	0.0028	0.638	0.0017

not statistically significant. Even with respect to the combined concentration of potassium and sodium, the difference between the plants from situations inside and outside the moat on Garden Key is not significant, as by the "t" test for $n=16$ the difference in question (0.009) corresponds to a value of P between 0.10 and 0.05. Although this would not justify a high degree of certainty that the combined potassium and sodium was significantly greater in the plants grown in the moat, the difference is suggestive, especially since it is parallel in direction to the observed difference of chloride concentration, which does satisfy tests that could not merely be due to chance.

The small differences of sap composition which exist between the *V. ventricosa* from the moat and that from the outer surfaces of its confining wall, therefore, suggest that the plants growing in the more sheltered situation attained the higher concentration of salts.

This contrast between the sap composition from plants grown on different sites, the salient features of which appear to be the degree of mechanical protection which they afford, may be more forcibly emphasized by further analysis of the data contained in table 10, sections I, II, III, and IV. It is significant that with respect to the mean chloride and potassium concentration in the sap sections I, II, III, IV present an ascending series. Even where the differences between adjacent members of the sequence are too small to be of statistical significance, they are still of interest in that the series does correspond qualitatively to one of increasing mechanical protection from surf. The relatively small plane surfaces of the pylons from which the cells of group I were obtained conceivably offered less protection than the interstices of the coquina rock which was the predominant substratum from which the cells of group II were obtained. Much more evident, however, is the fact that the massive confining wall of the moat presented, on its external eroded surfaces, cavities which added to the very considerable degree of protection already provided by the proximity and shape of the fort itself. Lastly, the plants collected from within the moat had a still more sheltered environment due to the confining wall itself, even though this was usually somewhat undermined in their immediate vicinity. Mechanical protection, therefore, emerges as one of the most significant of the factors which determine the composition of the sap of *V. ventricosa* in situ.

The habitats of sections I and II of table 10 were very similar with respect to the variable now under consideration, and moreover did not differ significantly with respect to the mean potassium content of the sap. For purposes of comparison, therefore, the data of sections I and II of table 10 have been combined and now comprise the "exposed group" of table 11, since the data they include correspond to the sap composition from collections made from sites which afford the minimum of protection from the mechanical and other effects of surf. Similarly section III of table 10 now constitutes the "partially protected group," and section IV, table 10, the "protected group" referred to in table 11. For further comparison a number of analyses of sap of *V. ventricosa* derived from plants originally collected on the sites named in sections I and II of table 10 but subsequently allowed to adjust to changed sea water under the quiet conditions of the laboratory (section V, table 10) comprise the group designated "collected plants" in table 11.

The mean values for the composition of the sap with their respective standard errors (table 11) indicate that significant differences exist between the "exposed," "partially protected," and "protected" type of habitat. This is confirmed by table 12, in which comparisons are drawn between the different groups and data are recorded which evaluate the significance or otherwise of the differences observed.

With respect to the three ions considered (K, Na, Cl) the differences between the "exposed" and "protected" habitats is significant, and it is clear that the sheltered environment produced a greater potassium concentration which was in part at the expense of sodium, but which was also accompanied by a significant increment of chloride concentration. It will be noted that the gain of chloride is almost balanced by the increment of potassium plus sodium in the "protected group."

The "partially protected" habitat of the outer sea wall of the moat of Fort Jefferson supplied plants which, with reference to their potassium content, were intermediate between the plants from the more extreme habitats included in the "exposed" and "protected" groups. The greater potassium and smaller sodium concentration observed in the "partially protected" as compared with the "exposed" groups are both statistically significant, but the chloride concentrations, which for all practical purposes may be regarded as the total salt concentration, of the two groups in question were the same. The differences, therefore, between the "exposed" and "partially protected" groups concern merely the relative amount of potassium and sodium in the cells. It appears that the greater shelter provided by the interior of the moat than by the seaward surface of the confining wall promoted, without loss of sodium, an enhanced total concentration of potassium chloride, of which the increase of chloride is significant. The same degree of certainty cannot be assigned to the corresponding gain of potassium (see comparison of "partially protected" and "protected" groups in table 12).

It is now of interest to compare the plants which were transferred from the habitats represented by sections I and II, table 10, to the laboratory, where they were allowed to adjust to changed sea water contained in large glass vessels. The vesicles formed a sparse, single layer in each dish, were subjected to the minimum of mechanical disturbance, and were exposed only to diffuse light of relatively low intensity. The outstanding fact is that this treatment caused an increase of potassium and chloride concentrations (in approximately equivalent amounts) to which high degrees of certainty may be attached, and this occurred without any decline in sodium concentration. The inevitable conclusion is that the cardinal feature of the transfer of the

TABLE 12

Groups compared	Ion	Difference between mean sap concentrations	Difference ÷ S.E. of difference	"t" test	Significance of observed differences
Exposed group: protected group	K..........	0.025	4.70	$t=4.8$; $n=25$ $\therefore P<0.01$	Significant
	Na.........	0.009	2.10	$t=2.0$; $n=25$ $\therefore P=0.05$	Just significant
	Cl..........	0.015	4.40	$t=4.4$; $n=25$ $\therefore P<0.01$	Significant
Exposed group: partially protected group	K..........	0.018	3.30	$t=3.09$; $n=23$ $\therefore P<0.01$	Significant
	Na.........	0.011	2.40	$t=2.27$; $n=23$ $\therefore P<0.05$	Significant
	Cl..........	0.002	0.56	....	Negligible
Partially protected group: protected group	K..........	0.007	1.16	$t=1.06$; $n=16$ $\therefore P=0.3$	Not significant
	Na.........	....	...	....	Not significant
	Cl..........	0.013	3.25	$t=3.18$; $n=16$ $\therefore P<0.01$	Significant
Exposed group: collected plants in laboratory	K..........	0.020	5.10	....	Significant
	Na.........	....	...	....	Not significant
	Cl..........	0.018	7.20	....	Significant
Protected group: collected plants in laboratory	K..........	0.005	1.04	....	Not significant
	Na.........	....	...	....	Not significant
	Cl..........	0.003	0.90	....	Not significant

plants from their substratum on the reef to the quiet conditions of the laboratory is the change from an exposed situation to one offering the maximum of mechanical protection, in which the plants are no longer exposed to the effects of surf. The relative unimportance of the light variable in this salt uptake was shown by the absence of any significant difference between plants in total darkness and others in diffuse light during this treatment in changed sea water.

It is, therefore, clear that the conditions of the laboratory approach those of the moat in so far as the sap composition of *V. ventricosa* is concerned. If the sap composition for the "protected" group and for that composed of "collected plants" (tables 11 and 12) are compared, it becomes apparent that the gain of potassium and of chloride which had occurred in the latter since they were collected raised the concentrations to a level at which they were not significantly different from the plants growing in the moat, in which the highest concentrations yet observed for cells in situ were found. On this basis the *V. ventricosa* as it occurs abundantly on Bird Key Reef at Tortugas, despite its very high concentration of salts, represents a population with a total salt concentration somewhat less than the maximum consistent with the stage of development of the vesicles, which therefore remain capable of some further slight salt uptake quite apart from the continuance of active growth and metabolism upon which salt accumulation commonly depends (80). Thus the subsequent uptake of salt which occurs in the quiet conditions of the laboratory and which appears to be independent either of any conspicuous growth, active metabolism, or even of light conditions, seems to be due to residual potentialities for salt accumulation which were determined by previous growth and not fully utilized on the reef. In the latter respect only, but not in its apparent independence of metabolism, this uptake of salt, though very much less in magnitude, resembles the extensive salt absorption which may occur when plants deprived of salt during development ("low salt plants"; see 30, 69, 80) subsequently have access to a salt supply and conditions conducive to absorption. *V. macrophysa,* on the other hand, exercises its maximum capacity for salt accumulation on the ledges of the moat and a decrease, not an increment, occurs when it is transferred and adjusted to the diffuse light and other conditions of the laboratory.[1] In view of recent evidence (44) that *V. macrophysa* as it exists at Bermuda responds to artificial light, this might represent a further avenue of approach to the laboratory study of this species at Tortugas.

The relation between the composition of the sap and the size of the vesicle from which it is obtained must be considered. This factor may be investigated with more confidence in *V. ventricosa* than in *V. macrophysa,* since the latter produces a dense mat of vesicles in which many of different sizes may be in intimate contact. In the case of *V. ventricosa,* which has the advantage of relative uniformity in the shape of the isolated single vesicles it produces, the difficulty is to exclude the other factors now known to influence the sap composition. Of these, the habitat factor was surmounted by restricting the collections for this purpose to as limited an area as possible. A single tide-filled pool at the seaward edge of Bird Key Reef and a limited area on the seaward surface of the confining wall IV of the moat on Garden Key were selected for this purpose, and comparisons were made only between collections secured on the same day at about mean low water when the plants were still submerged. This practice limited the total number of plants available, and consequently the composition of the sap at any given size of vesicle could be estimated only from a single pooled sap sample. This unfortunately does not permit the effect of variability of the plants to be evaluated at each size level chosen, a formidable task if all other factors are to be eliminated, and, therefore, the relationship between size of vesicle and composition of sap must rest rather upon the consistent graded series obtained than upon a strict evaluation of the significance of the differences between adjacent members of the size series. However, it seems that complete confidence may be placed in the relationship revealed, since it can be derived from two entirely independent series of observations, one made in 1933, the other in 1934, and also receives confirmation from a similar series of samples derived from plants which had remained in sea water in the laboratory for several weeks.

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[1] The reader is reminded that a similar situation may obtain at Bermuda, since the potassium concentration of the "seasoned cells" is commonly lower than that which was obtained from the collections of Crozier (23).

After collection and prior to sap extraction, the vesicles were graded according to their diameter, and the average weight or volume per vesicle for each size group was obtained by weighing in a tared beaker of sea water after the surface water had been removed. The weight or volume data are used directly as the criterion of size, and any small concomitant differences of density are neglected. The data obtained are given in table 13 and illustrated in figure 4.

There is clearly a tendency, reflected by the chloride data, for the total salt concentration to decline as the size of the vesicle increases. Assuming that size reflects age (see p. 106) and state of development, this series is in accord with a somewhat general principle according to which the youngest, most actively growing cells and organs have the greatest power of salt accumulation (69, 70, 80). The possibility that this effect is due merely to the relative decrease of surface which accompanies increase of volume seems to be remote. A calculated correction factor based upon the relative proportion of surface to volume when applied to the total chloride concentration, to the excess of chloride concentration over that in Tortugas sea water (0.570 M.), or to the chloride accumulation ratio fails to compensate the differences determined by size of the vesicle.

The evidence available indicates that the size factor operates both upon the total salt concentration and upon the potassium concentration. In a given habitat the variability with respect to potassium concentration is always greater than that encountered for chloride (see table 11). This is no doubt determined by slight variations in the magnitude of those factors responsible for the larger differences between the groups shown, but, whatever its origin, it renders the comparison with respect to potassium in the size series more difficult than that for chloride. Even so, the well-marked drift of chloride concentration with size is also accompanied by a similar

Table 13—*The effect of the size of the vesicle on the sap composition of Valonia ventricosa*

(Sap concentrations in gm. equivs. per liter)

Collection details	Size data		No. of vesicles in sample	[Cl]	[K]
	Average diameter (cm.)	Average weight (gm.)			
1933, Bird Key Reef	...	0.43	50	0.632	
	...	0.86	35	0.628	
	...	1.50	20	0.624	
	...	2.90	9	0.620	
	...	4.60	6	0.611	
	...	7.90	2	0.609	
1934, Garden Key, outer sea wall of the moat	...	0.70	20	0.637	0.617
	...	2.50	8	0.627	0.593
	...	9.00	4	0.617	0.590
	...	18.00	1	0.611	0.570
1934, collected from Bird Key Reef, kept in aquarium with slow stream of sea water	1.20	...	24	0.654	0.616
	1.75	...	7	0.639	0.604
	2.06	...	4	0.639	0.609
	2.70	...	3	0.635	0.595

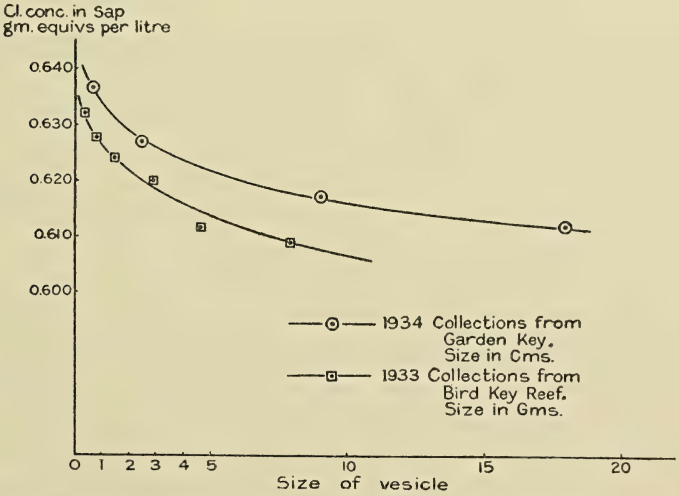

Fig. 4—Sap concentration in relation to size of vesicle, *Valonia ventricosa.*

one for potassium concentration. Other factors than size enter into the comparison of the data derived from these special collections designed to reveal the effects of size with the mean values for the same habitat given in table 10. Even so, it is clear that the extreme values of chloride and potassium concentration in the size series of table 13 deviate from the mean value recorded for the location from which the collections were made (see table 11) by amounts which approach the level of statistical significance, although when tested by the table of "x" (Fisher, 26, table 1) the deviations yield values of P which are greater than 0.05.

In the case of *V. ventricosa*, therefore, it is evident that in addition to the habitat factors already stressed, there is the additional one of size or age. It is necessary here to stress the point that although the data upon which habitat factors were assessed could not of necessity be confined to vesicles strictly of the same size, this does not vitiate the conclusions drawn. The effect of size becomes conspicuous only when the extremities of the size series are compared. The mutually compensating effect of these on the mean composition of sap samples from random collections is further much reduced by two factors: first, the relative infrequency of the largest vesicles (see fig. 2), and second, the tendency of the collector to reject the small ones, which are difficult to remove from their substratum and which contribute but little to a sample of sap. Since the figures included in the regional survey were almost invariably derived from composite sap samples (as only the largest single plants provide sufficient sap for analysis), it is of interest to form an estimate of the size range which, with due regard to the interaction of the two factors of frequency and volume per vesicle, in a composite sap sample from a random collection of plants contributes most to its bulk. From the data of table 3 it may be calculated that the size range from approximately 2.0 cm. to 1.2 cm. diameter would contribute about 50 per cent of the total sap obtained.

COMPARISON OF THE FACTORS WHICH DETERMINE SAP COMPOSITION AND OCCURRENCE

Within the range of conditions which *V. macrophysa* will tolerate at Tortugas, the dominant factor which determines the composition of its sap seems to be that of light. In the case of *V. ventricosa*, the factor of mechanical protection or exposure to surf is the predominant one. It is of interest to compare the factors which determine distribution and abundance with those which determine the sap composition of the species in question. In the summary table 1, the sites recorded for *V. ventricosa* are in descending order of exposure to, and increasing order of protection from, the effects of surf and currents. It has also been shown that they correspond to an ascending order of salt accumulation in the plants they produce. The corresponding frequency of occurrence cannot be compared on the basis of quantitative data, but there can be no question that the abundance is greatest on Bird Key Reef and not in those sites where the salt concentration was greatest, namely, certain parts of the moat of Fort Jefferson where the frequency of occurrence was so low that prior to 1934 (83) the organism had not been recorded in the moat. These facts may be explained if the distribution of *V. ventricosa* is regarded as a compromise between a sufficient degree of mechanical protection to permit the vesicles to exist and acquire the salt concentrations necessary for growth, and freedom from those marked diurnal fluctuations of temperature, acidity, and oxygen content which are typical (53, 73, and table 2 of this paper) of an isolated body of water (e.g., the moat) heavily colonized with algæ and which *V. ventricosa* apparently will not tolerate.

Valonia macrophysa, on the other hand, is relatively tolerant of considerable diurnal fluctuations in acidity, oxygen content, and temperature of its environment and may even exist where these are extreme (e.g., moat pools; see p. 104), but it is completely intolerant of the effects, presumably mechanical, associated with surf. Plants collected from the ledge of the moat and placed in large glass flasks, perforated with six to eight holes and suspended at a depth of several feet from a buoy moored some 50 yards from the laboratory dock in a position not exposed to violent currents, failed to survive more than a few days. It will be found by comparison of table 9 and the summary of distribution in table 1 that the milieu which produces the greatest abundance of this species is also the one which is associated with the highest salt concentration (ledge of moat walls VI, II, and III). However, the mere conditions of light, temperature, composition of sea water, etc., which are conducive to high salt content do not suffice to produce a luxuriant growth, for these properties also obtain on certain of the inner fort walls (e.g., III, II), which, because they fail to provide the necessary physical conditions yielded by the horizontal ledge, never support a luxuriant stand. It must not be forgotten that *V. macrophysa*, like *V. ventricosa*, does not appear early in the colonization of a naked surface, and the extent to which the growth and nutrition of both forms are dependent upon association with other species represents a factor which is difficult to evaluate, but which may be very real and assume considerable importance when the contrast between the heavily colonized ledge and the relatively bare surface of the inclined fort wall is recalled.

SUMMARY OF SECTION III

Valonia macrophysa—Differences occur in the composition of the sap of *V. macrophysa* grown in different parts of the moat of Fort Jefferson.

The principal causal factor appears to be the light condition which obtains during growth.

The data available fall into three well-defined groups, and the significance of the differences between them can be evaluated.

The best light condition (longest exposure to light of highest intensity) produced the greatest potassium and lowest sodium concentration in the sap. The total salt concentration was not significantly affected.

If *V. macrophysa* is dissected into single vesicles after it is collected, its potassium concentration falls and sodium concentration rises. Subsequently in sea water and diffuse light under laboratory conditions recovery takes place. During recovery potassium is absorbed, and this will occur during some days even in sea water of reduced potassium concentration. It is believed that this explains the claims of Brooks (13).

The new steady level of potassium concentration attained under laboratory conditions is significantly lower than that of the plants in situ in the moat.

Valonia ventricosa—The principal causal factor which determines the sap composition of *V. ventricosa* during growth is the degree of shelter and protection it obtains. The information available does not permit the effect of light to be evaluated.

Differences with respect to potassium, sodium, and chloride concentration occur between the three groups into which the available data fall. The significance of these differences can be estimated.

The most sheltered environment (the moat) produces the highest potassium, lowest sodium, and highest chloride concentration.

Vesicles transferred from the reef to the quiet of the laboratory increase in concentration of potassium and chloride.

The new level of sap composition thus attained does not differ significantly from that observed in the plants from the moat.

There is a relation between the sap of *V. ventricosa* and the size of the vesicle. The smallest, and presumably the youngest, vesicles have the highest potassium and chloride concentration. This is in harmony with a general tendency for the accumulating power of plant cells to decline with age.

General—The conditions which produce the most abundant growth of *V. macrophysa* likewise produce the greatest concentration of potassium and lowest concentration of sodium. The subtle variables which determine growth transcend the effect of the potassium concentration or pH of the medium in their effect upon the potassium concentration of the sap of *V. macrophysa*, and the variables known to operate upon the sap composition of *V. ventricosa* in situ are equally independent of the possible variations of potassium or hydroxyl ion concentration to which this organism is subjected in its habitat.

IV. EXPERIMENTAL CONDITIONS WHICH MODIFY THE SAP COMPOSITION OF VALONIA

The range of sap composition of *V. macrophysa* and *V. ventricosa* in situ at the Dry Tortugas has been described and some attempt made to specify the causal factors. The effect of certain variables upon the composition of the sap of collected plants under laboratory conditions must now be discussed.

The factors most amenable to experimental variation are those which refer to the external medium. Normal sea water may be modified with respect to the most important ions, namely, K, Na, Cl, H, and OH, and from the effect induced in the sap their relative importance as determinants of its composition may be assessed.

The limitations of this method are obvious. It utilizes relatively mature vesicles, removed from their habitat and all those intimate environmental associations which alone permit these highly specialized organisms to flourish. With reference to *V. macrophysa,* Osterhout (62) has added the further complication that only experiments performed at Bermuda in the winter can be viewed with favor! The use of collected plants under laboratory conditions and seasonal cycles, if real, may tend to reduce their activity and, therefore, minimize behavior due to the processes of growth.[1] However, in evaluating much of the recent theory concerning the mechanism of the entry of salts into *Valonia,* this limitation is not serious. The theories in question do not entail so intimate a relationship between the vital properties of the living system and the uptake of salt and maintenance of high internal concentrations as that which emerges from work on more active systems (4, 30, 69, 74–80, 84).

In all cases of salt accumulation the obvious difficulty is that during entry, the movement is not in accord with an ionic concentration gradient. For the case of potassium uptake by *Valonia,* Osterhout circumvented this difficulty by a method now too well known to require more than passing mention (58, 59). In brief, Osterhout adopted an expedient and thereby retained the simple view that entry is determined by the magnitude and direction of a concentration gradient. This was rendered possible by the now familiar thesis that the ions of electrolytes cannot penetrate the protoplasm, so that entry of solutes is confined to undissociated molecules. This conception was originally designed to account for the rate of penetration of weak acids (H_2S, H_2CO_3; see 56, 65), weak bases (NH_4OH; see 22), and dyes (see papers by Irwin cited by Osterhout [58, 59]); but it was later concluded that entry of potassium and sodium is confined, not to molecules of KCl and NaCl, but only to undissociated molecules of free base, that is, KOH and NaOH respectively. It was suggested that the magnitude and direction of the gradient of concentration of "undissociated free base" determines the movement of K or Na, a gradient which can be evaluated in terms of the ionic products K×OH and Na×OH, the so-called "thermodynamic potentials of free base." It is to be noted that according to this view the living system plays no more intimate part in salt accumulation than to provide the membrane system with the requisite properties. Of these, impermeability to ions, a greater preference for undissociated molecules of base than of neutral salts, and a surprisingly greater degree of permeability to KOH than to NaOH are outstanding. The measure of justification which these ideas possess they derive rather from the study of model systems in which mixtures of phenols play the imagined rôle of the protoplast than from crucial evidence that the membrane in vivo really possesses these attributes.

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[1] The inadequacy of sluggish extension as evidence of the capacity to grow has been dealt with earlier.

Respiration and metabolism, on the other hand, are assigned but a minor rôle in salt accumulation. The former appears only as a potential source of carbon dioxide, the function of which is to regulate acidity (59, p. 1003), and what reference has been made to other metabolic processes has largely centered around other hypothetical acid products of metabolism, designated HA, which could play a similar part (58, 59). Granted the living system with the properties assigned to it, entry of potassium involves movement along a positive gradient of the mobile component and would ultimately result in equilibrium. Growth, or more strictly extension, becomes the resultant of entry of salt, an osmotic response to high internal salt concentrations. Clearly on this view the impetus for salt entry does not derive from the ability of the plant to grow, but resides in diffusion gradients which can be calculated from the composition of sap and external medium. The theory stands or falls according as the evidence supports or refutes the claim that the system tends to diffusion equilibrium between sap and solution with reference to the quantities designated "undissociated free base" (i.e., K×OH, Na×OH). The changes in the composition of the sap which ensue in sea water modified as to its composition permit this thesis to be tested.

It is important to recognize that nothing in the theory of Osterhout limits it to plants at any particular season of the year or to those which are actively growing. Plants which have ceased growth should the more quickly reach "equilibrium" under any given conditions. Entry of water merely defers the attainment of diffusion equilibrium with reference to the mobile "molecules of free base." While extension may greatly increase the quantities absorbed, the theory is based upon gradients of concentration and must ultimately be evaluated upon the basis of *concentrations and not total amounts.* Whenever the sap composition responds to changes in the composition of the external solution, these should be in accord with the theory if it is valid. This emphasis is necessary because whenever comparisons are drawn between the work done at Bermuda and elsewhere the seasonal issue is invariably raised and attention is diverted thereby from the principal points at stake.

It has been shown that collected specimens of *V. macrophysa* and *V. ventricosa* attain characteristic levels of concentration in sea water under laboratory conditions. The changes in sap concentration which are experimentally produced must, therefore, be measured from the concentration levels characteristic of the species adjusted to laboratory conditions. The data available characterize this sap composition and permit an estimate to be made of the significance of any given experimentally induced deviation from the mean sea-water value.

TABLE 14—*The range of sap composition of Valonia in changed sea water under laboratory conditions*

Species	Ion	Mean conc. (gm. equivs. per liter)	S.D. (gm. equivs. per liter)	S.D. (% of mean)	S.D. of mean (gm. equivs. per liter)
V. ventricosa	K.......	0.599	0.0092	1.54	0.0023
	Na......	0.044	0.0071	16.10	0.0018
	Cl.......	0.638	0.0069	1.09	0.0017
V. macrophysa	K.......	0.497	0.0105	2.12	0.0035
	Na.......	0.123	0.0153	12.26	0.0052
	Cl.......	0.622	0.00606	0.97	0.0020

The requisite data are to be found in table 14. Adopting the usual convention, single observations which depart significantly from the mean sea-water value must do so by amounts not less than twice the corresponding standard deviation. From the value of this deviation in terms of the standard deviation $(\frac{x-m}{\sigma})$, the probability P that the difference in question could have occurred by chance can be estimated (26, p. 44). For comparative purposes it is frequently convenient to express all sap concentrations relative to those which obtain in the sea-water controls, which are arbitrarily designated 100. The standard deviation is, therefore, given both in absolute units and on the relative basis as a percentage of the mean.

THE EFFECT OF [K], [Na], and [Cl]

There were two ways in which the external solution was modified with respect to potassium, sodium, and chloride concentration. In the first (series I of table 15), standard volume molar solutions of potassium or sodium chloride were prepared in sea water. By this means the cation and chloride concentration and also the total osmotic pressure were increased. Tortugas sea water had a chloride concentration of 0.570 M. and a potassium concentration of 0.011 by direct analysis. Solutions with the compositions shown in table 15, nos. 1 to 5, were made by this procedure. It will be noted that the relative variation is greatest in the case of potassium, since solution 1 had a potassium concentration 5.5 times solution 3, while the changes in sodium and chloride concentration and osmotic pressure seem comparatively small.

TABLE 15—*Composition of sea water modified with respect to K, Na, and Cl*

Series	No.	Solution	[Cl]	[K]
I	1............	0.05 M. KCl in sea water	0.620	0.061
	1a............	0.03 M. KCl in sea water	0.600	0.041
	2............	0.01 M. KCl in sea water	0.580	0.021
	3............	Sea water	0.570	0.011
	4............	0.01 M. NaCl in sea water	0.580	0.011
	5............	0.05 M. NaCl in sea water	0.620	0.011
II	6............	1 vol. KCl 0.570 M. to 4 vols. sea water	0.570	0.122
	7............	1 vol. KCl 0.570 M. to 10 vols. sea water	0.570	0.062
	8............	Sea water	0.570	0.011
	9............	1 vol. NaCl 0.570 M. to 10 vol. sea water	0.570	0.010
	10............	1 vol. NaCl 0.570 M. to 1 vol. sea water	0.570	0.005

The second procedure (series II) preserved a constant chloride concentration and simultaneously provided the maximum range of potassium and sodium concentrations. Stock potassium chloride and sodium chloride solutions having the same chloride concentration as sea water were prepared in distilled water and these mixed in the stated volume proportions with sea water to give the solutions numbered 6 to 10 in table 15. It is to be noted that in series I the solutions at the extremes (nos. 1 and 5) are slightly hypertonic[1] to sea water (no. 3), and in series II the extremes (nos. 6 and 10) are slightly hypotonic to sea water (no. 8). In salt absorption experiments an exaggerated importance may easily be attached to small variations in osmotic pressure. This often occurred in the older literature of culture solutions; the tendency was no doubt the heritage of the long preoccupation of plant physiologists with the problems of plasmolysis. Brooks (13) studied the behavior of *V. macrophysa* in solutions of varying composition with respect to potassium and sodium, and concentrated upon the attempt to maintain their osmotic pressure constant. Misadventure rendered most of these precautions abortive, so that the experiments were actually performed under conditions neither of constant halide concentration nor of constant osmotic pressure. In the experiments to be described, the effect of variation in external potassium and sodium concentration can be assessed at constant chloride concentration and also for solutions enriched with salt. The results show that the concomitant variations in osmotic pressure did not obscure the principal conclusions which emerged. To facilitate comparisons between the two series and the two species, the data for final sap composition are given upon a relative basis in table 16. These are calculated relative to the sea-water value as 100. The sodium values are omitted, since the greater variability with respect to this ion rendered all the differences insignificant.

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[1] The increment of osmotic pressure was less than 1 per cent.

TABLE 16—*Composition of the sap of Valonia ventricosa and V. macrophysa in sea water modified with respect to K, Na, and Cl*
(Sap concentration in sea water=100)

Series	No.	Relative final sap concentration			
		Experiment A *V. ventricosa*		Experiment B *V. macrophysa*	
		[K]	[Cl]	[K]	[Cl]
I	1	110	107	...	...
	1a	...	...	108	108
	2	102	101	104	102
	3	100	100	100	100
	4	100	100	102	102
	5	102	104	101	105
II	6	103	101	101	100
	7	98.7	100	102	101
	8	100	100	100	100
	9	100	101	102	100
	10	100	99.6	93.2	98.6

The experiments were carried out in large pyrex glass beakers and the solutions were gently agitated once in twelve hours. All the cultures of a given series were covered by a large bell jar. The initial weight of each culture was obtained. The graded, surface-dried vesicles[1] were weighed in a tared beaker of sea water. The final weights invariably showed a slight increase (order of 5 per cent), but the effect of the treatments upon this was insignificant. Experiment A lasted five days and experiment B lasted six days.

The data show (table 16) that when sea water was modified with respect to potassium and sodium but at constant chloride concentration (series II), there was no significant effect upon the chloride concentration of the sap despite the wide range of potassium and sodium concentrations in the external solutions (see table 15). Similarly the effect upon potassium concentration was negligible. Only the decrease of potassium concentration which occurred in the *V. macrophysa* exposed to solutions very low in potassium and high in sodium (series II, experiment B, no. 10) can be regarded as significant, and this may merely mean that injury occurred in these solutions.

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[1] The drying was accomplished by placing carefully on a thick, soft Turkish towel and allowing to drain for a few seconds.

Since all the solutions used had the same pH as sea water, the conclusion from series II is that neither of the species responded in the manner expected on the assumption that the composition of the sap is determined by diffusion gradients calculated in terms of the ionic products K×OH and Na×OH. The same conclusion would apply to gradients of potassium ion concentration or to gradients in terms of the product K×Cl.

In series I, on the other hand, the gain of chloride concentration which occurred in the sap of plants adjusted to the solutions enriched with potassium or sodium chloride is significant. This is true of both species (table 16). At the extremes of the series the differences can be tested as in table 17, and it may be noted that the intermediate members of the series were also intermediate in chloride concentration. The gain of chloride in the solutions enriched with potassium chloride was greater than in those enriched with an equimolar concentration of sodium chloride. The fresh weight data, as well as the relative uptake of potassium and chloride, showed that the concentration changes observed could not have been due merely to the movement of water.[1] The solutions enriched with potassium chloride produced a highly significant gain of potassium in the sap of both species, while in those solutions to which sodium chloride was added and in which there was a significant gain of chloride concentration, the gain of potassium which occurred was not significant.

TABLE 17—*Significance of the effects on sap composition induced by changes in external solution*

Expt.	No.	Ion	$\frac{x-m}{\sigma}$	P	Significance of result
A	1..........	Cl	6.4	Very small	Highly significant
	5..........	Cl	3.7	0.0001	Highly significant
	1..........	K	6.5	Very small	Highly significant
	5..........	K	1.3	0.19	Insignificant
B	1a.........	Cl	8.3	Very small	Highly significant
	5..........	Cl	5.2	Very small	Highly significant
	1a.........	K	3.8	0.0001	Highly significant
	5..........	K	0.5	0.61	Insignificant

x=observed value.
m=mean for sea-water control.
σ=standard deviation of population in sea water under laboratory conditions.
P=probability that difference observed could have occurred by chance.

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[1] It is really immaterial how the concentration changes were produced, whether by movement of salt or of water; the point at issue is their relationship to concentration gradients of K×OH.

Brooks (16) still believes that *V. ventricosa* at Tonga in sea water modified by mixture of isotonic sodium chloride shows a relative gain of potassium. This conclusion rests on one culture and one time period. The principal result of these experiments of Brooks seems to be the relative lack of response in sap concentration to changes of external potassium concentration. Jacques and Osterhout (43) describe the changes which occurred in *V. macrophysa* in sea water and sea water mixed with artificial "sea water" which had higher halide concentration than the normal (*loc. cit.*, table 11). Despite this, both the control and the low-potassium sea-water (0.006 M.) cultures showed a progressive loss of potassium and chloride. The significance of the small difference between these rates of decline seems to be a matter for statistical estimation, though it is presumed to be real. The cultures enriched with potassium remained steady in potassium concentration after three days. The slight gain observed cannot have been greater than from 0.475 to 0.487 (mean of data at 3, 6, 10, 15, 20 days). Lacking a measure of variability in the initial material, this gain is in doubt. The principal result of Jacques and Osterhout's experiment seems to be the relation of the sap, not to external potassium concentration, but to the variable which caused "cells" in sea water to decline in potassium even though the concentration of this ion in the external medium remained constant. This variable was not specified more than by the statement that "it was presumably due to the conditions of illumination and temperature."

The evidence of experiments A and B shows that at Tortugas both species tolerate wide changes in external potassium and sodium concentration without significant effect upon their sap composition, but that they readily respond to small increments in external concentration of potassium when this is accompanied by increased chloride concentration. These facts indicate that the ionic products K×OH and Na×OH do not have the importance which has been attached to them, and that, on the contrary, the dominant ion is chloride. Unless the conditions permit simultaneous uptake of chloride and potassium, increase of external potassium concentration does not cause entry of this ion.[1] When entry of potassium does occur, it is regulated rather by the need for equivalence with chloride uptake than by the actual external potassium concentration. This is shown by the approximate equality in the gain of potassium and chloride from the solutions enriched with potassium chloride, although the relative increase of external potassium concentration was very much greater than that for chloride (table 15). This is shown in table 18, and for this comparison the absolute units (gm. equivs. per liter) are used.

The basis for the preference for potassium rather than sodium to accompany chloride cannot be discussed at length. It seems, however, more reasonable to seek the explanation in terms of the ions as Brooks has suggested (15) than in terms of the molecules KOH and NaOH. This involves the vexed problem whether the membrane in vivo approximates to a pore system, or to a non-aqueous film, or combines these properties. It is not a new suggestion that the similarity of mobilities of the potassium and chloride ions is the basis of their approximately equal uptake. Though the differences of mobility may be greatly exaggerated by membranes of the pore type (for a recent review of this subject see 36), this may still prove to be the most profitable line of approach despite the criticism which the idea has received (59, p. 1004). Osterhout and Stanley (68) claim that even though the order of cation penetration approximates to that of ionic mobilities in water, this does not constitute an insuperable difficulty, on the theory that the membrane is non-aqueous and penetration is in molecular form (KOH, NaOH). They show that for a particular non-aqueous substance the same order is obtained for the partition coefficients between water and non-aqueous phase. The obvious advantage of the present tendency to combine the pore and solubility theories is that it permits the rapid penetration of the nonpolar compounds to be interpreted, but it does not exclude the entrance of water-soluble substances, and if these are in the form of charged ions the very slowness of their penetration is also intelligible. All such views may bear upon the relative amounts of potassium and sodium which are absorbed, but they leave the ultimate problem of the accumulation of salt unexplained. For this neither pore or solubility membranes are adequate except in so far as they form part of a vital system which is capable of metabolism.

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[1] The proportion of K to Na in the sap is somewhat determined by the conditions and especially by the pretreatment. The K/Na exchange which may occur during adjustment from one environment to another is not in question here.

TABLE 18—*Gain of K and Cl in the sap of Valonia from sea water enriched with KCl*
(Sap concentrations in gm. equivs. per liter)

Series	Expt.	No.	Species	[K]−[K] in sea-water control	[Cl]−[Cl] in sea-water control
I	A	1	*V. ventricosa*	0.058	0.042
I	B	1a	*V. macrophysa*	0.043	0.049

THE EFFECT OF [H] AND [OH]

This aspect of the problem assumes especial importance because of the suggestions of Osterhout (58, 59), Brooks (13), and Briggs (8). *Valonia* is outstanding because it accumulates its salts from a medium very much more alkaline than the sap. It is this unusual property of *Valonia* and its environment which has become the cardinal feature of the theory of Osterhout, although among flowering plants it would represent the exceptional rather than the general condition. It is, therefore, necessary to inquire whether the composition of the sap is regulated by the pH relations of sap and external solution. The pH relations between vacuole and external solution are usually most easily modified by controlling the external pH. The solutions from which absorption commonly proceeds in plants are of low salt concentration and amenable to the use of buffers, for example phosphate or bicarbonate. Sea water is, however, a complex system the reaction of which is already regulated by the CO_2/HCO_3 buffer system. The mere addition of acid or alkali is valueless as a means of regulating its reaction during periods long enough for absorption of alkali halides to occur. The sap, however, unlike that of most higher plants, is but weakly buffered and can be modified by the rapid entrance of weak bases. Therefore, the earlier work on this subject, from which the theory of Osterhout emerged, was concerned mainly with the effect of a reduction of the pH gradient from sap to external solution by the use of ammonium chloride externally. It is unfortunate that the movements of potassium and sodium caused thereby were with, not against, the prevalent ionic gradients, and that they therefore admit of the alternative explanation that in the presence of ammonium, owing to injury or any other cause, potassium and sodium responded to these gradients. Later this aspect will be re-discussed in the light of experiments at Tortugas, but it is evident that particular importance must be attached to those more difficult experiments in which the changes induced in the sap are related to the pH of the external medium. The effect of external pH upon the rate of penetration of certain weak bases and acids (NH_4OH, H_2CO_3) has played no small part in the development of the theories mentioned (22, 42, 58, 59, 65). However, these experiments were confined to very short periods (in some cases as short as a few minutes), and frequently accumulation was never attained. Recently, and since the experiments at Tortugas were performed, Osterhout has described experiments which purport to demonstrate an effect of external pH upon the entry of potassium. These utilized partly those limited and uncontrolled changes of external pH due to the metabolism of the organism and also a technique based on the addition of acid or alkali to sea water (see 44, p. 730).

THE EFFECT OF EXTERNAL [H] AND [OH]

The experiments to be described utilized the evident fact that the only effective way to control the external pH effectively is to make use of the CO_2/HCO_3 buffer systems already available in sea water. The only component of this which admits of experimental variation to produce and maintain a wide range of reactions is the partial pressure of carbon dioxide with which the sea water is in equilibrium. Sea water, unmodified in its salt content but in equilibrium with carbon dioxide-free air, yields a reaction as alkaline as pH 9.0 to 9.2, whereas normally at Tortugas the reaction of the laboratory sea water in equilibrium with air had a pH of 8.3 to 8.4. If the pressure of carbon dioxide is increased, more acid reactions can be produced, and reactions as acid as those of the sap (pH 5.5) can be obtained without altering other sea-water constituents. For this purpose facilities which had been developed at Tortugas for a study of the respiration and metabolism of *Valonia* by methods comparable to those used by the writer for storage tissue (75) were utilized. Compressed air was obtained from a motor-driven, automatically regulated compressor fitted with storage tank and reducing valves which supplied a manifold from which, through fine-adjustment needle valves, air could be drawn at rates accurately controlled. Five air lines were operated in parallel and each was equipped with a calibrated flow meter with water manometer and a train of wash bottles and pressure stabilizer (for details see 75). For the most alkaline reaction air was delivered to the *Valonia* cultures after all the carbon dioxide had been removed by washing in alkali and the air stream passed through carbon dioxide-free sea water in a Drechsel wash bottle. The appropriate indicator was added to the final wash bottle, and this gave warning if the desired conditions were not being obtained. Air not washed in alkali, but only in sea water, produced a steady pH of 8.3. The air enriched with carbon dioxide was obtained by controlled mixing of the air and carbon dioxide streams, each of which was kept steady by reducing valves from the high-pressure supply and recorded by the flow meter. The controlled mixing was effected by adjustments of needle valves on the arms of a Y-tube attached to the sources of air and carbon dioxide respectively. The approximate volume proportions of air and carbon dioxide could be obtained from the readings of the flow meter and the final adjustment was obtained empirically, after which the various valves were locked to deliver these amounts permanently. Indicator, added to the final sea-water wash bottle, warned if any unforeseen variations occurred, but actually the system operated faithfully and the only limitation to this technique was the amount of carbon dioxide required. The method was used both continuously and intermittently. The continuous method was adopted in 1933. The *Valonia* was contained in large four-liter blackened bottles containing two liters of solution, fitted with gas inlets and outlets which were immersed in a large, mechanically stirred thermostat in which a steady flow of sea water produced a very stable temperature. This type of experiment was limited, mainly by the quantity of carbon dioxide required, to a period not exceeding two days. In 1934 the same procedure for the production of the desired reactions was adopted, but the cultures were equilibrated to the gas mixtures only twice daily. In the interim they were securely stoppered and placed in the dark. The drift which occurred was slight, as the combined gas and liquid volume in the containers provided considerable inertia. In all cases the actual reactions of the solutions were determined colorimetrically either by comparison with buffer standards or by use of a Hellige-Klett comparator loaned by Dr. Graves.

It was evident from experiments A and B that *V. ventricosa* and *macrophysa* which had adjusted to sea water and the conditions of the laboratory could be induced to increase their concentration of potassium and of chloride in solutions enriched with potassium chloride. This procedure was, therefore, applied to the experiments to ascertain the effect of the reaction of the external medium on the entry of potassium and chloride into the sap. The concentrations used were 0.025 M. KCl and 0.05 M. KCl prepared in sea water. The effect of the pH alone upon the composition of the sap was assessed in sea-water cultures not enriched with potassium chloride, and by comparison with corresponding cultures enriched with salt the combined effects of the variation in pH and increased concentration of potassium chloride were assessed. When the continuous method of applying the CO_2/air mixtures was used (experiment C), the sea-water controls and the cultures enriched with salt which received the same gas treatment were connected in series and the gas stream was rapid enough to be unaffected by its passage through a single culture.

In the first experiment (1933, experiment C) two series were used. Series I consisted of sea water containing *V. ventricosa* random sampled from a graded, uniform population adjusted to sea water and the conditions of the laboratory. Series II was similar in every respect except that the sea water was enriched with 0.05 M. KCl. The data obtained are given in table 19.

TABLE 19—*Experiment C. Effect of external* pH *on sap composition of Valonia ventricosa in sea water and sea water plus 0.05 M. KCl*

External pH	Relative sap concentrations *			
	Series I Cultures not enriched with KCl		Series II Cultures enriched with 0.05 M. KCl	
	[K]†	[Cl]	[K]†	[Cl]
5.8.......	99.5	98.0	103.5	99.0
6.8.......	100.0	98.3	103.5	100.0
8.3.......	100.0	100.0	103.5	101.0
9.2.......	100.0	98.6	103.5	100.0

* Relative to the sap of vesicle in sea water at pH 8.3.

† The K determinations were done both by the cobaltinitrite method and later by the chloroplatinate method. Each set of data leads to the same conclusion, but the figures given were calculated from the chloroplatinate results.

To facilitate comparisons, the data are expressed on a relative basis by setting the value in sea water in equilibrium with air at 100.

It is evident that in both series (I and II) the *V. ventricosa* tolerated for the period of the experiment, admittedly short, a very wide range of external pH (actually more than a thousand-fold variation in [OH]) without significant effect on the potassium content of the sap. On the other hand, the relatively small difference of potassium and chloride concentration between series I and II caused by the addition of 0.05 M. KCl (i.e., less than a sixfold increment of K) caused a consistent increase of K in the sap. This increase (3.5 per cent of the mean sea-water level) was too large to have occurred by chance (P between 0.02 and 0.03). Since no real difference exists between the members of the same series (see table 19), the significance of the gain of potassium concentration due to the increased potassium chloride concentration can be estimated by the "t" test.[1] If so, the difference between the means of series I and II equals 19 mgm. equivs. per liter; $S = 2.08$, therefore $t = 12.9$, whence for $n = 6$, P is very small, since $t = 3.7$ when $P = 0.01$. Therefore a very high degree of certainty can be assigned to the observed increase of potassium concentration in series II as compared with series I. A similar calculation for chloride yields P between 0.1 and 0.05, a value which indicates that the gain of chloride is on the borderline of statistical significance. Owing to the much greater variability with respect to sodium (see table 14), all the differences of sodium concentration observed were insignificant and the figures are, therefore, omitted from table 19.

The result then is clear. The sap responds more readily to comparatively small variations in potassium and chloride concentration than to relatively large variations in hydroxyl ion concentration. A significant response with respect to potassium in the sap was obtained by an increase of external potassium concentration to about 5.5 times its normal value, but in the same period of time the sap did not respond significantly to a variation of hydroxyl ion concentration of over 1000 times. On the theory that the "gradients of undissociated free base" determine the sap composition, the terms K and OH are of equal importance in the product $K \times OH$. Clearly this is not true. The composition of the sap is more intimately related to the concentration of potassium and of chloride than it is to that of hydroxyl, the effect of which appears to be small. In this respect the effect of pH confirms the conclusion derived from experiments in which the concentrations of potassium, sodium, and chloride were the variable factors, and the combined results cast the gravest doubts upon the validity of the view that the sap composition is determined by gradients calculated in terms of the products $K \times OH$ or $Na \times OH$.

The result of experiment C was of such importance that the whole procedure was repeated in 1934. Only *V. ventricosa* rigorously graded by size of the vesicle was used in experiment D, and in experiment E only *V. macrophysa* derived originally from healthy stands with large regular vesicles from the ledge of wall VI of the moat. In order that these experiments might last longer, the solutions were equilibrated to the CO_2 air mixtures by the intermittent method. Otherwise the procedure was similar to that described for experiment C.

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[1] Calculation carried out in absolute units, not the relative figures of table 19.

The results of experiment D, which lasted four days, are given in table 20. Series I represents *V. ventricosa* cultures in the sea water unmodified with reference to potassium chloride and at the reactions specified; series II differed only in that the sea water was enriched with 0.025 M. KCl; and in series III the added concentration was 0.05 M. KCl. All the saps were first analyzed at Tortugas and the analyses subsequently repeated at Berkeley. Since the potassium was determined gravimetrically by the chloroplatinate method in the later analyses, these figures are to be preferred (though the Tortugas data lead to the same conclusion) and are, therefore, used in table 20. It is to be regretted that two members of series II were not available for analysis at Berkeley, but the remnants of the series suffice to show that series II yielded figures intermediate between series I and III. Sodium was determined in all cases, but again it was found on analysis of the figures that all of the differences with respect to this element were insignificant, again owing to the relatively great variability normally encountered, and therefore they are omitted from the table.

TABLE 20—*Experiment D. Effect of external* pH *on the composition of the sap of Valonia ventricosa in sea water and sea water enriched with KCl*

(Duration of treatment 4 days)

External pH	Relative sap concentration *					
	Series I Sea water		Series II Sea water plus 0.025 M. KCl		Series III Sea water plus 0.05 M. KCl	
	[K]	[Cl]	[K]	[Cl]	[K]	[Cl]
8.6–8.8	103	100	...	...	110	106
8.0–8.2	100	100	...	...	111	108
7.0–7.2	101	100	106	104	117	114
6.0–6.3	101	101	107	104	111	108
5.4–5.6	96.5	98.5	102	102	107	104

* Concentration relative to the sap of vesicles in sea water in equilibrium with air=100.

Experiment D, therefore, fully confirmed the results obtained from experiment C. Despite very drastic changes in the external concentration of hydroxyl (e.g., pH 8.7 to 6.2), the sap retained a constant composition (series I). Only when the external pH was reduced close to the value of the sap did some loss of potassium and chloride occur. It is noteworthy that at this very acid external reaction the effect on the sap was not large even after several days. By this time some of the vesicles were beginning to show signs of injury, and it seems that the slight change of sap concentration which occurred was the result, certainly not the cause, of this injury. Until irreversible effects intervene, the direct results of changes in hydroxyl ion concentration upon the potassium and chloride *concentration* of the sap of *V. ventricosa* in sea water are negligible.

On the other hand, the differences of potassium and chloride concentration in the sap of corresponding members of series I and III are all highly significant; so much so that it is superfluous to estimate the probability that they could have occurred by chance. Again, therefore, although but little response was obtained to a very wide range of [OH] in sea water unmodified with respect to potassium chloride, a very definite increment both of potassium and of chloride was obtained in the cultures enriched with a relatively small concentration of potassium chloride. Again it appears that the internal potassium concentration is more intimately linked to the concentration of potassium and chloride than to hydroxyl. According to Osterhout's original view (later somewhat modified by subsidiary hypotheses), the uptake of potassium is determined by the gradient of the ionic product K×OH, and this should be a maximum at the most alkaline external reaction. A clearly marked maximum of both potassium and chloride uptake appeared, not at pH 8.7, but at pH 7.1. Still more pertinent is the evident gain of potassium and chloride in the most acid culture of series III over its corresponding member in series I. This increment of concentration occurred at a reaction such that the difference of acidity between sap and solution was negligible. It must not be supposed that the pH of the sap was very much reduced. No reaction more acid than pH 5.7 was recorded on the freshly extracted sap. M. M. Brooks (11) recorded that saps from cultures enriched with carbon dioxide were more *alkaline* than normal after the carbon dioxide had been removed from the sap. This has been observed by Fife in plants which are capable of the hydrolysis of amide to ammonia.[1] There were indications of similar effects on the pH of the sap from *Valonia* exposed to sea water enriched with carbon dioxide in our experiments. However, the potassium data for series I did not show a significant gain of potassium in the acid solutions, which was the explanation postulated by Brooks.

A further experiment (E, table 21) similar to experiment D was carried out using *V. macrophysa*. At very low pH (e.g., 5.4 to 5.6) this organism[2] is much more susceptible to irreversible injury than is *V. ventricosa*. Previous experience also suggested that it was more liable to injury when the sea water was enriched with 0.05 M. KCl. In the experiment (E) to be described, the latter condition proved serious only in the most alkaline culture, which was accordingly abandoned, but the former prevented any data of value being obtained in the solutions at pH 5.4 to 5.6. Series I consisted of the control cultures not enriched with potassium chloride, series II consisted of those containing a supplementary concentration of 0.025 M. KCl, and series III involved the sea-water solutions containing an addition of 0.05 M. KCl. The duration of the experiment was four days. The solutions were equilibrated to the gas mixtures by the intermittent method. All the saps were analyzed at Tortugas and subsequently at Berkeley. The latter analyses were the more reliable. In series III, the relative sap concentration of two of the samples recorded can be calculated only from the analyses done at Tortugas. The close agreement between the figures calculated from both sets of data for the culture at pH 8.3 to 8.4 shows that no serious error is incurred by their inclusion. Although sodium was determined on all saps, the sodium figures are omitted from the table since they do not depart significantly from the mean sea-water value.

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[1] J. M. Fife and V. L. Frampton, The effect of carbon dioxide upon the pH and certain nitrogen fractions of the sugar beet plant, Jour. Biol. Chem., vol. 109, 643–655, 1935.

[2] Also noted at Bermuda.

TABLE 21—*Experiment E. Effect of external* pH *on the composition of the sap of Valonia macrophysa in sea water and sea water enriched with KCl*
(Duration of treatment 4 days)

External pH	Relative sap concentration *					
	Series I Sea water		Series II Sea water plus 0.025 M. KCl		Series III Sea water plus 0.05 M. KCl	
	[K]	[Cl]	[K]	[Cl]	[K]	[Cl]
8.6–8.8	103	99.7	106	103	. . .	. . .
8.3–8.4	100	100	108	106	111 † 111 ‡	107 108
7.0–7.2	100	100	106	105	107	106
5.9–6.1	100	102	102	103	107	105

* Relative to concentration in sea water at pH 8.3 to 8.4 as 100.
† Analyses at Berkeley. K by chloroplatinate method.
‡ Analyses at Tortugas. K by cobaltinitrite method.

In series I, despite a range of external pH from 8.7 to 6.0 and a period as long as four days, no significant effect on the potassium or chloride concentration in the sap occurred. In series II, however, the increment of potassium and of chloride concentration is significant (except at pH 6.0), although the cultures at pH 7.1 and 8.7 do not deviate significantly from that at 8.3 to 8.4. Comparing series III and series I, the increases of potassium and chloride concentration, which occurred in the former at the three reactions for which data are available, are highly significant, and it is noticeable that the differences which were due to the increase of total potassium chloride concentration much exceed the relatively small, and perhaps not significant, differences within each series which were due to the variation in the concentration of hydroxyl.

Therefore the conclusions which were derived from the experiments on *V. ventricosa* in solutions of different pH (and which were summarized on page 142 may be extended to *V. macrophysa.* In both cases the concentration of potassium chloride in the sap proved independent of large changes in the concentration of hydroxyl but was intimately related to the concentration of potassium and chloride and responded to relatively much smaller changes of these constituents. From experiments A and B it seems that, of the factors in the external solution, the chief emphasis should be placed upon the chloride concentration and not upon the concentration of hydroxyl or the product K×OH. The failure to recognize the importance of the chloride ion in the past was probably due to the fascination of the absorption or accumulation ratio, which for chloride is not impressive.

Since these experiments were completed the results of Jacques and Osterhout (44) have become available. Comparison was made of the potassium concentration and total potassium content ([K]×vol.) of *V. macrophysa* in sea water more alkaline (pH 8.8) and more acid (pH 6.8) than normal. Experiments were done in the dark, in artificial light, and in diffuse daylight with flowing cultures. The first two methods yielded negative effects of pH whether regarded from the standpoint of the concentration or of the total content of potassium in the sap. The experiments in diffuse daylight were also negative in so far as the relation between external pH and concentration in the sap was concerned. An attempt was made to establish the reality of the effect of pH upon the total potassium uptake by utilizing the volume data. Some of the small differences require more intensive analyses in terms of the variability and the errors of the product [K]× vol. before much credence can be given to them. It must be stressed, however, that there is in these results no indication that the pH of the external solution caused differences of *concentration* in the sap. If under the conditions of the Bermuda experiments the control of external pH determines growth and thus leads to slight differences of total potassium content, then there is no more reason to suppose that the changed value in the product K×OH is the determining factor than that the effect upon growth per se is the one responsible.

Jacques and Osterhout (45, 45*a*) examined the effect of external pH upon potassium uptake by *Nitella flexilis* Ag. Quite contrary to their conclusions on *V. macrophysa,* they find that no significant effect of pH upon potassium uptake and potassium absorption occurred in defiance of the gradient K×OH. It may be concluded with safety that a similar result would have been obtained with almost all fresh-water organisms or higher plants, even as the writers contend that it also follows from experiments on *Valonia.* Jacques and Osterhout (45*a*) cling to the idea of concentration gradients, and in the endeavor to find a suitable gradient of an undissociated molecule turn to K×HCO_3. At the same time they reach the conclusion, which all along has been self-evident, that the uptake of salt by *Nitella* requires energy. The conclusion that energy is required was reached by Jacques and Osterhout only upon a priori grounds. The evidence is to be found in the experiments of Hoagland, Davis, and Hibbard (32, 33, 34), which show the effect of light. Jacques and Osterhout, however, have been able to derive their conclusion despite the fact that their own experiment showed that the potassium uptake that they were dealing with occurred equally in the light and in the dark; the conditions of oxygen supply and the carbohydrate content of the plants were not described by Jacques and Osterhout. Little importance need be attached to the disparity between the earlier results of Hoagland and Davis and those of Jacques and Osterhout until the latter authors evaluate the significance of the pretreatment of their experimental material. After it was removed from the ponds, no doubt at some distance, the plants were washed and stored in artificial solution "in the cold from a few days to two weeks, the dead cells being removed at intervals." The presumption is that this treatment caused an initial loss[1] of salt content from that which prevailed in the pond or the maximum the cells attain, and produced a condition resembling that of "low salt" plants (30, 69, 80), which under more favorable conditions of light, temperature, and salt supply absorb salt despite the absence of external signs of growth. Until the living materials which are used in salt absorption experiments are evaluated in these terms, comparisons between different investigations are futile and general conclusions dangerous.

The Effect of Internal [H] and [OH]

The experiments already described which were concerned with the effect of variations in the pH of the external medium on the sap of the two species available at Tortugas produced results which were not in accord with prevalent theories of the mechanism of salt accumulation in *Valonia*. Some experiments were, therefore, made to determine the effect of changes in the internal pH. This approach to the problem is the more interesting because it was adopted earlier by Osterhout, and the results then obtained, if they did not actually inspire the theory that gradients of "thermodynamic potential of undissociated free base" determine penetration, have at least been regarded as its best confirmation (58, 59).[2] The experiments made at Tortugas comprised an attempt to verify these results.

Experimentally (42) Osterhout utilized the familiar fact that weak electrolytes commonly penetrate cells more readily than strong. In sea water plus ammonium chloride the ammonium penetrated and rendered the sap more alkaline, and the subsequent effect upon the concentration of potassium and sodium constituted the chief point of interest. The data were harmonized by Osterhout with the demands of his theory and he showed that the calculated gradients of $K \times OH$ and $Na \times OH$ were consistent with the entrance of sodium and exit of potassium. This treatment appears to offer a striking confirmation of the postulates of the theory. It is, however, unfortunate, as emphasized by Steward (79, 80) that a theory which purports to explain the great accumulation of potassium and the relative exclusion of sodium should be tested by a treatment which actually produced the changes of potassium and sodium concentration in the converse direction and by data which can be interpreted in different ways. The obvious explanation that the concentration changes of potassium and sodium were directly due to the injurious effect of ammonium and not merely to its effect upon the pH relations of sap and external medium, though emphatically revived by Steward (79), has been equally emphatically denied by Osterhout (62). It is to be noted that toxicity of ammonium is a common experience with plants and that ammonium is usually tolerated only by those plants which are rich in carbohydrate and have an extensive organic acid metabolism, properties which no one has yet shown that *Valonia* possesses.

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[1] The subsequent loss in distilled water under experimental conditions may have some significance in this connection.

[2] See also Bot. Rev., vol. 2, 283–315, 1936, where many of the same data are reproduced.

The experiments of Jacques and Osterhout (42) concerned the behavior of *V. macrophysa* in sea water plus 0.001 M. NH_4Cl. The experiments at Tortugas were mainly done with *V. ventricosa* but were verified for *V. macrophysa.* The concentration of ammonium chloride in the Tortugas experiments was the same as in those from Bermuda.

A special population of *V. ventricosa* was obtained and adjusted for three weeks to laboratory conditions in changed sea water in shallow glass vessels and in darkness or diffused light of low intensity. The vesicles were rigorously graded according to size and, for experiment F, divided into two samples of 65 vesicles each, of diameter ranging from 0.5 to 0.75 inches. It must be emphasized that these specimens were originally as healthy and perfect as it is possible to obtain. To one dish was added 0.001 M. NH_4Cl in sea water ("NH_4 group"), and the second contained an equal volume of sea water ("sea-water group"). The cultures were side by side in almost total darkness, and the solutions were changed daily without undue agitation. It was quickly apparent that the "NH_4 group" were showing visible effects due to the treatment, of which the first, an appreciably lighter color than the controls, appeared less than 72 hours after contact with the ammonium salt. From this time onward the symptoms became progressively more extreme and involved a steadily increasing number of vesicles. After 4 days many specimens showed a marked irregularity of their chloroplasts and a tendency for the disorganized plastids and protoplast to aggregate, producing a characteristic granular appearance. The formation of viable "aplanospores," a not infrequent response to injury both mechanical and chemical, was not observed. Finally the now almost colorless plants became progressively less turgid until after 11 days not a single specimen of the "NH_4 group" remained completely unaffected, although the "sea-water" controls did not deviate in any visible way from their original condition. Prior to 11 days several comparable samples of sap had been obtained from both groups, but obviously injured plants were not included in these. After 11 days the 39 remaining vesicles of the "NH_4 group," originally 65, were divided for sap analysis into three subgroups. In the first subgroup were 12 vesicles which were losing their turgor and showed the maximum disorganization of the chloroplasts (11 [I], table 22); the second subgroup included 13 specimens in which the chloroplasts had almost disappeared but the turgor persisted (11 [II], table 22); and finally the third subgroup, numbering 14 (11 [III], table 22), comprised those in which the turgor and color most nearly approached to normal. The analytical data obtained are given in table 22.

TABLE 22—*Experiment F. The effect of 0.001 M. NH_4Cl on the sap composition of V. ventricosa*

(Sap concentrations in gm. equivs. per liter)

Time (days)	NH_4 group					Sea-water group			
	pH	[K]	[Na]	[NH_4]	[Cl]	pH	[K]	[Na]	[Cl]
0.......	...	0.597	0.035	.....	0.630	...	0.597	0.035	0.630
2.......	...	0.594	0.033	.....	0.630	...	0.599	0.038	0.630
4.......	6.5	0.591	0.035	0.003	0.630	5.9	0.591	0.052	0.636
6.......	6.9	0.592	0.041	0.007	0.636	5.7	0.599	0.036	0.636
8.......	7.2	0.462	0.173	0.005	0.633	5.7	0.602	0.033	0.639
11 I..	7.9	0.025	0.612	0.002	0.614				
11 II..	6.8	0.482	0.162	0.005	0.635	5.7	0.590	0.047	0.639
11 III..	6.6	0.584	0.054	0.014	0.644				

Throughout the period of 11 days the sap composition of the "sea-water group" remained unchanged (table 14 indicates what constitutes a significant difference). Turning to the "NH_4 group" at 11 days when the entire group was affected in some degree, it is clear that the entry of sodium and exit of potassium is determined by the severity of the symptoms of injury, which cannot be deduced from the internal ammonia concentration. The cation content of the sap of subgroup I was principally sodium, although the potassium concentration still exceeded that of the external solution. A remarkable fact is that the vesicles of subgroup I showed a sodium concentration actually higher than that of the sea water. This condition is without parallel in normal *Valonia,* but is met with in *Halicystis Osterhoutii* at Bermuda. A significant decline in chloride concentration and the lower ammonium concentration of this than of the other two subgroups obviously indicates that the sap of the most severely affected vesicles was fast approaching equality of concentration with the external medium with reference to the various sap constituents, including hydrogen ion. In subgroup II, in which the injury symptoms were less severe, the sap had declined in potassium concentration and increased in sodium concentration by amounts which are highly significant. In subgroup III, which was visibly affected by the presence of ammonium, the decline in potassium concentration approached the level of significance ($P=0.10$), though the increase of sodium concentration, relatively large though it appears to be, commands a smaller degree of certainty. It is of some interest that in those vesicles which were least affected by the presence of the ammonium and in which the ammonium concentration was still high, the chloride concentration was also greater, though not significantly so, than in subgroup II and the sea-water controls.[1]

Clearly the changes of potassium and sodium concentration which are induced in the sap of *V. ventricosa* by the presence of ammonium chloride in the external medium are the result of, and do not precede, the injurious and irreversible effects produced by prolonged contact with this substance.

Experiment F was conducted in the dark. The possibility that the effect of ammonium might be different in the light was considered. A long "cradle" to carry stoppered bottles, filled without air space with the desired solution, was constructed and provided with suitable weights, buoys, and anchorage so that it could be submerged about 3 feet below the water surface above a white patch of coral "sand." By this means high light intensities were obtained without exceeding the temperatures which *V. ventricosa* resists with impunity at Tortugas. Forty-six comparable specimens of *V. ventricosa* selected from a large population of 600 specimens which had adjusted to laboratory conditions were placed in bottles containing sea water and sea water plus 0.001 M. NH_4Cl respectively. When these were submerged, as described, a certain amount of mechanical agitation was inevitable and in both groups some vesicles succumbed. The experiment (G) was discontinued after 3 days and sap was obtained from mechanically uninjured specimens of both groups with the results shown in table 23. During the three days of this treatment a loss of potassium and gain of sodium (the former significant) occurred. This was identical in both the groups and was due, not to the presence of ammonium chloride, but to the transfer from the quiet conditions of the laboratory (see pp. 123–125). The interest of these figures is that although the ammonium-treated cultures showed an uptake of 0.013 M. per liter of sap and had a pH more alkaline by 0.4 units than

TABLE 23—*Experiment G. Effect of 0.001 M.* NH_4Cl *in the light on the sap composition of Valonia ventricosa*

(Sap concentrations in gm. equivs. per liter)

	[K]	[Na]	[NH_4]	[Cl]	pH
Sea-water group	0.575	0.056	.....	0.631	6.1
NH_4 group	0.577	0.056	0.013	0.645	6.5

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[1] There is here some suggestion of a tendency to increased osmotic pressure in presence of ammonium chloride which would cause greater extension. Jacques and Osterhout (42) refer to the fact that *V. macrophysa* treated with NH_4Cl "grew" better than in sea water.

the controls, yet their potassium and sodium concentration was unaffected, at least until 3 days, by which time the prominent symptoms referred to in experiment F had not developed. It is also evident that the chloride concentration of the "NH_4 group" exceeded that of the "sea-water group" by an amount which balanced the ammonium absorbed, although neither deviated from their original laboratory sea-water value (table 14) by amounts which can be regarded as significant.

These results, so far as they go, obtained from experiments in the light and at the temperature of the sea, are consistent with the conclusions drawn from experiment F, namely, that the effects of the presence of ammonium chloride upon the concentration of potassium and sodium in the sap of *V. ventricosa* are secondary and are due to the onset of injurious and irreversible effects.

In the ammonium chloride experiments, Osterhout used *V. macrophysa.* The only query which remains is whether under Tortugas conditions the effect of ammonium chloride upon *V. macrophysa* would be different from its effect upon *V. ventricosa.* Time permitted but a single experiment (H) to determine this. From the vesicles which did not succumb to injury the data shown in table 24 were obtained. Again it was found that for relatively short periods appreciable concentrations of ammonium in the sap were tolerated without decline in potassium concentration, but that after more prolonged contact the loss of potassium and gain of sodium occurred, though not in such extreme degree as in the most severely affected specimens of *V. ventricosa* (table 22, 11 days).

TABLE 24—*Experiment H. Effect of 0.001 M. NH_4Cl on the sap composition of Valonia macrophysa*

(Sap concentrations in gm. equivs. per liter)

Group	[K]	[Na]	[NH_4]	[Cl]
Sea-water control	0.494	0.133	.....	0.631
Sea water plus 0.001 M. NH_4Cl (3 days)	0.498	0.127	0.006	0.623
Sea water plus 0.001 M. NH_4Cl (10 days)	0.454	0.167	0.009	0.623

These experiments, therefore, justify the criticisms which have already been made (79, 80) and which are reiterated here, that the experiments involving the effect of ammonium chloride upon *Valonia* and resulting in a decline of potassium concentration and increase of sodium concentration cannot supply a conclusive proof of the theory of Osterhout, especially as it applies to strong bases. Such proof demands a crucial test based upon technique which produces positive effects, that is, significant increments of potassium concentration in the sap. The variables which ought to produce such increments have been examined as already described, but the experimental observations did not accord with these theoretical expectations. All experience at Tortugas with the two species available there in quantity justifies the inference that the effects of ammonium which are significant are secondary to the injury which this substance causes. Osterhout has replied (62) that at other seasons of the year and at Bermuda different results are obtained with *V. macrophysa*. It has been shown in an earlier section how ill-founded are some of the views which have been expressed concerning the physiological status of *Valonia* at Tortugas in the summertime. It is recognized that there are factors which regulate the severity of the injury caused by ammonium, as shown by the variation between the three subgroups I, II, and III in table 22. Granted that the different conditions to which Osterhout refers may have mitigated the most extreme symptoms of injury seen in *V. ventricosa*, one cannot evade the criticism that the effects of ammonium chloride upon the concentrations of potassium and sodium were of the kind to be expected from injury. Since the injury has been amply demonstrated for the Tortugas material and it has been admitted that it also occurs at Bermuda at other seasons of the year, the degree of injury which obtained in the crucial experiments under discussion becomes of the first importance. This, unfortunately, does not admit of quantitative estimation in the case under discussion, since the very effect upon potassium and sodium concentration which is in dispute represents the only accurate criterion available. A test of viability and vigor which has often been advocated for *Valonia*, namely, the absence of sulphate from the sap, is valueless. Sulphate penetrates so slowly that sap from plants visibly affected and even suffering loss of turgor frequently failed to give a positive test for sulphate. The only conclusion which is possible in the light of the experiments here described is that those experiments which are concerned with the effect of the internal pH of the sap upon its composition and which involve ammonium are inadmissible, since the effects due to the use of ammonium are complex and not directly attributable to its effect upon internal pH.[1] They do not constitute unequivocal proof of the theory they have been supposed to support. This theory must, therefore, seek its justification elsewhere, and since this can hardly rest upon the effects observed in the sap by modifications of the composition of the external solution, which lead to conclusions in opposition to it, its further utility even for the interpretation of the special case of *Valonia* seems slight. All recent results indicate that when applied to living systems other than *Valonia* the theory has to contend with even greater difficulties and can be upheld only by subsidiary and arbitrary assumptions (62, 69) which rob it of any hope of general application. Since the phenol models are designed to illustrate the theory and embody the chief points which are here disputed, they lose even that little significance which systems so far removed from physiological reality could possibly possess for the problem in vivo.

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[1] Since the preparation of this paper the work of L. R. Blinks has appeared (Jour. Gen. Physiol., vol. 19, 633–672). In this (pp. 659–661) reference is made to certain complex effects of ammonia upon the bioelectrical properties of *Valonia*.

SUMMARY OF SECTION IV

Experiments to determine the variables which affect the sap composition of *V. macrophysa* and *V. ventricosa* under laboratory conditions are described.

The significance of differences obtained experimentally is estimated from the variability in the sap concentrations of potassium, sodium, and chloride met with in sap samples derived from populations adjusted to the conditions of the laboratory.

Both *V. ventricosa* and *V. macrophysa* gain significantly in potassium and chloride concentration in sea water enriched with small concentrations (0.05 M. and 0.025 M. respectively) of potassium chloride. Equal increments of sodium chloride produce significant but smaller increments in the sap, but neither the observed gain of potassium nor of sodium is significant. At constant chloride concentration both species can be exposed to a wide range of external potassium and sodium concentration without significant effect on the sap concentration of potassium, sodium, or chloride.

The dominant external factor is the concentration of chloride. The gain of chloride in the sap is accompanied by an equal gain in potassium concentration despite the great disparity in the relative increase of their external concentrations. The potassium concentration of the sap is not determined directly by either the concentration of potassium ion or the product $K \times OH$, but is closely related to the external chloride concentration.

Methods are described whereby the pH of sea water can be regulated for relatively long periods.

In sea water the responses of *V. macrophysa* and *V. ventricosa* to wide variations in external hydroxyl concentration are negligible. In sea water enriched with potassium chloride the responses to relatively small increments of potassium and chloride are real and significant even at reactions so acid that they approach that of the sap. In the case of *V. ventricosa* a significant maximum of potassium and chloride uptake occurred at pH 7.0.

These experiments concerned with the external pH agree with those concerned with the variation in K, Na, and Cl concentration and indicate that the sap concentration is independent of the gradient in the products $K \times OH$ and $Na \times OH$ but is sensitive to the total concentration of potassium chloride.

An examination of the effects of changes in internal pH, secured by the use of ammonium chloride externally, upon the concentration of potassium and sodium in the sap leads to the conclusion that these are secondary to the irreversible effects which this substance produces. The effects of modifications in internal pH accord rather with the view that, until the direct effects upon the living system are evident, these plants tolerate considerable change of their internal pH without loss of potassium or gain of sodium.

The emphasis recently placed upon gradients of undissociated free base (K×OH and Na×OH) between sap and sea water is misplaced. The relation between potassium absorption and chloride concentration is closer than the relation between potassium absorption and hydroxyl concentration.

The previous examination of the effect of habitat factors upon the sap composition showed that the important variables are intimately concerned with the activity of the living system which is reflected in the growth and distribution of the organism, and that these transcend the effect of those very small changes of potassium, or hydroxyl, concentration which can possibly occur in the sea. The results of experimental alterations of potassium concentration and pH similarly minimize their importance. Within the limits consistent with the development of the organism, the total salt concentration of the sap is regulated by the chloride concentration of the external medium. There is no reason to suppose that the properties which regulate the relative proportions of potassium and sodium in the sap are not mutual properties of the ions and the living system.

V. THE METABOLISM OF VALONIA IN RELATION TO SALT ACCUMULATION

All vacuolated plant cells which have been investigated hitherto readily accumulate the bromide ion if they retain some ability to grow or possess an enhanced capacity for rapid salt uptake caused by a minimal salt supply during previous growth (4, 69, 70, 32, 33, 34). It is also evident that the cells most active in growth and metabolism are most effective in the accumulation of the bromide ion (4, 69, 70, 80). Even the relatively mature internodal cells of *Nitella* accumulate considerable bromide accompanied by potassium, although most of the bromide they absorb is at the expense of chloride already present. However, repeated attempts to induce *Valonia* to accumulate bromide from sea water plus potassium bromide all failed, whether the experiments were conducted in the light or in the dark.[1] Hitherto the only cells from higher plants which had failed to accumulate bromide were those which had irreversibly lost their earlier powers of active growth and division, and frequently also their ability for protoplasmic streaming (4). On this basis, therefore, *Valonia*[2] would be comparable rather with these less active systems than with the cells at the apex of a growing root (69) or shoot (70, 80), or at the surface of thin disks of tissue from certain storage organs (76, 4). In the case of *Valonia,* however, the failure to accumulate bromide may be due in part to the interference between the absorption of chloride and bicarbonate ions with bromide. Such inter-ionic effects have been observed with other cells (35). Although bromide could not be demonstrated in the sap, gains of potassium and halide concentration occurred which suggested that the *Valonia* responded to the increased concentration of potassium and total salt as it would have done in sea water enriched with potassium chloride. The data for two experiments (I and J) are given in table 25. Of these, one (J) was conducted in the quiet conditions of the laboratory, the other (I) on the submerged cradle already described (p. 150).

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[1] This failure is the more surprising since bromine owed its discovery to its relative accumulation in the ashes of seaweeds. Höber and Höber (37) observed that in strong bromide solutions (sea water plus isotonic bromide), bromide displaced chloride but equality of concentration was not attained. Höber and Höber worked with *V. macrophysa* at Naples.

[2] Protoplasmic streaming in *Valonia* only occurs conspicuously in the rhizoidal cells (25).

TABLE 25—*Sap composition (gm. equivs. per liter) of Valonia ventricosa exposed to sea water and to sea water enriched with KBr*

(Duration of experiment 11 days)

Experiment	Solution	Conditions	[K]	[Cl]+[Br]
I	Sea water	Light	0.579	0.635
		Light	0.582	0.630
		Dark	0.586	0.630
	Sea water plus 0.025 M. KBr	Light	0.606	0.654
		Dark	0.607	0.654
J	Sea water	Dark	0.591	0.639
	Sea water plus 0.025 M. KBr	Light	0.607	0.648
		Dark in equilibrium with N_2	0.609	0.654

Table 25 shows that there was no significant difference between cultures in the light and in the dark if the external solutions were the same. In experiment I the sap from *Valonia* in sea water enriched with 0.025 M. KBr showed a significant gain of potassium and of halide concentration over the sea-water controls, which, as expected, declined somewhat in potassium concentration from the value typical of the still conditions of the laboratory. The results of experiment J carried out in the laboratory are similar; though lacking the replicates in the sea-water controls, the differences are somewhat less significant since the sap from plants in the bromide sea water did not deviate significantly from the range which *Valonia* tolerates under these conditions in sea water. Experiment J is quoted mainly because it shows that cultures of *Valonia* not only retain their existing potassium and chloride concentrations under the low oxygen conditions provided by periodic equilibration to cylinder nitrogen, but even gain in salt content under the influence of an added external concentration. In this respect experiment J confirms another experiment of the preceding season in which various oxygen mixtures were continuously employed without any significant effect upon the sap.

These experiments emphasize the striking difference between *Valonia* and other plants, for example *Nitella* (32, 33, 34) and *Elodea* (70), and tissue from various storage organs (74, 77, 82) and roots (30, 82). Not only did *V. ventricosa* fail to accumulate bromide, but its potassium and halide *concentration* in sea water and sea water enriched with potassium salts are independent alike of light and of low oxygen content. The information available suggests that *V. macrophysa*, like *V. ventricosa*, does not accumulate bromide under experimental conditions.

The effect of temperature was not examined at Tortugas. Osterhout (59, 60) stated that in a dark ice box the potassium concentration of *V. macrophysa* decreased. This cannot be admitted as evidence that the potassium concentration decreases with a lower rate of metabolism, because metabolism was not measured. At this extremely low temperature irreversible effects on the living system are to be expected with an organism commonly found in warm seas, and these would lead to the same result. Analogy with known temperature effects on metabolism would here be dangerous, since temperatures in the vicinity of zero frequently increase carbon dioxide production in plants which contain starch. With reference, therefore, to the most important variables (light and oxygen concentration), which for other plants have suggested the relation between metabolism and salt accumulation, *Valonia* yielded only negative results under experimental conditions. A direct comparison between the metabolic activity of *Valonia* and other plants which have figured prominently in salt accumulation experiments, therefore, became necessary. The property upon which this comparison could be based most conveniently was the respiration rate.

The direct measurement of the carbon dioxide output in marine algæ presents difficulties not encountered with a plant in fresh water. Although respiration data (oxygen uptake and carbon dioxide production) are available for other marine algæ (38),[1] direct measurements which indicate the order of magnitude involved in the case of *Valonia* seem to be lacking.

Lacking facilities for the use of manometric methods and also anticipating a low respiration rate, a method which permitted large quantities of *Valonia* to be employed for long periods of time was first employed. This was based on that used for storage tissue (75) and involved the use of an aeration system with absorption towers to absorb carbon dioxide, which was then determined by titration methods. To overcome the difficulty that sea water itself when equilibrated to carbon dioxide-free air yields carbon dioxide, different procedures were tried. The sea water was adjusted to carbon dioxide-free air before the *Valonia* was inserted, and in other experiments a sea-water control was used. It soon transpired that the carbon dioxide obtained from sea water alone was of the same order as that from sea water plus *Valonia* even when the amounts of the latter were of the order of 100 gm. in less than 1 liter of sea water. No method of reducing the sea-water blank by sterilizing it proved practicable. Finally all the carbon dioxide collected from a strict sea-water control, during the experimental period and also after it was acidified at the end of this period, was compared with the total carbon dioxide obtained from the sea water plus *Valonia* during long periods in the dark, to which was added the remnant obtained from the acidified sea water. During 1933 this procedure merely indicated that the rate of carbon dioxide output was so small that not even its order of magnitude could be determined with certainty. In 1934 the experiments were repeated. The continuous aeration procedure was abandoned. The estimate of respiration was based on a comparison of the carbon dioxide released by acid and aeration from equal volumes of the final external solution and from a blank sea-water culture. The experiments were conducted in stoppered bottles filled with sea water previously equilibrated to air. The differences between sea-water control and *Valonia* culture were small and erratic and merely proved again that the output of carbon dioxide by sea water plus *Valonia* was not significantly greater than that of sea water alone. It therefore still remains to be critically established that carbon dioxide is evolved by *Valonia* in the dark. These experiments, however, do place an upper limit upon the possible respiration rate. As the sap from plants in the dark may become slightly more acid, although Crozier (23) stated that it remains constant, any carbon dioxide produced may await in this fluid the return of light and conditions for photosynthesis. In theoretical speculations concerned with the case of *Valonia* (58, 59, 8, 13) it has been tacitly assumed that carbon dioxide is produced, bicarbonate ion exported, and the sap maintained as if in equilibrium with a continuous supply of carbon dioxide. Direct attempts to measure the carbon dioxide evolved do not inspire confidence in these too facile assumptions. Osterhout may have experienced a similar difficulty, for, although carbon dioxide figures prominently in the phenol model systems, the acid produced by the metabolism of *Valonia* is not actually specified; in the theoretical treatment it is merely designated HA, which includes hypothetical organic acids, though the implication is that it is carbon dioxide.

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[1] The rates of respiration may be high; for example, that of *Ulva lactuca* is of the order of 0.12 mgm. CO_2 per gram fresh weight per hour. The respiratory quotient of the green algæ approximated to unity. This paper also described the effects of the salt content of the external medium on the respiration of marine algæ, many of which showed an increased rate in response to a decreased salt content.

The only other evidence of the evolution of carbon dioxide by *Valonia* in the dark is that indirectly derived from the decrease of external pH following periods of photosynthesis (44) in closed bottles of sea water. The increase of pH in the sea water in the light is familiar and easily demonstrated, and was described by Crozier (23). As far as the writers are aware, there are no data which permit the respiratory rate to be derived in absolute units from the observed pH changes, nor even conclusive proof that these were due to the loss of carbon dioxide by the *Valonia* vesicles.

The carbon dioxide production of *Valonia* is, therefore, of an entirely different order of magnitude from that of those other tissues already referred to, with which it has been proved that the rate of respiration is linked with salt accumulation. In fact it is a real problem to decide whether the magnitude of the output of carbon dioxide would suffice for the rôle which has been assigned to it in the salt absorption of *Valonia*. This can only be determined by simultaneous measurements of respiration and salt uptake, measurements which involve great difficulty in the case of these organisms.

Notwithstanding the doubts which still remain concerning the carbon dioxide production of *V. ventricosa,* its ability to absorb and produce oxygen may be demonstrated with ease. One set of figures is given in table 26; other data were obtained both in 1933 and in 1934 with similar results. The submerged "cradle" described earlier, provided with a cheesecloth screen to reduce somewhat the otherwise very great light intensity, was utilized. Selected *V. ventricosa* vesicles were placed in 550-cc. bottles and the bottles filled full of sea water (about 500 cc.). After suitable periods the solutions were analyzed for dissolved oxygen by the Winkler method.

TABLE 26—*Oxygen absorption and production by Valonia ventricosa*
(Solution, sea water. Total hours, 72)

No. of vesicles	Total final weight (gm.)	Conditions	Final solution dissolved O_2 (mgm. per liter)
None	....	Light	3.75
43	33.6	Light	18.20
43	34.6	Dark	2.45
42	34.8	Dark (lab.)	1.15

The solutions were sampled for analysis at 2:45 p.m. The oxygen concentration in the light culture was, therefore, the result of photosynthesis during this day and the excess of photosynthesis over respiration on preceding days. The figures indicate that *V. ventricosa* is far more active in photosynthesis than in respiration. This conclusion, which likewise emerged from other similar experiments, represents a curious reversal (8, 13) of an earlier erroneous deduction from the conditions of its habitat. The conclusions from these figures of oxygen production receive further support from the observations of Doyle, who showed that the plastids of *Valonia* may be plentifully supplied with small starch grains (25). In view of this undoubted activity in photosynthesis, the absence of any effect of light upon the concentration of sap constituents, or absorption from an external solution, such as that observed for *Nitella* (32, 33, 34) and *Elodea* (70), has an added interest. In the case both of *Elodea* and of *Nitella* it is clear that light exerts its influence upon salt absorption by means of its effect upon the concentration of sugar in the cells and of oxygen in the medium. The oxygen uptake of *Valonia* is so low that its further limitation due to the reduced oxygen concentrations which develop in closed containers in the dark is improbable. Similarly, since photosynthesis normally exceeds respiration, the system should be adequately stored with the products of photosynthesis to withstand relatively long periods of darkness. If, as Osterhout claims, light can determine the total *quantity* of salt absorbed (in contrast to the *concentrations* attained) because it also regulates extension, then the emphasis must be upon the effect of light on the processes of growth and metabolism, and not merely upon the relatively small changes in the gradient of pH between sap and medium which light can produce. In this respect *Valonia* would more nearly resemble other green organisms.

The absorption of oxygen by *V. ventricosa* in the dark is beyond dispute. It corresponds, however, to a rate of oxygen absorption which is very small. Only the order of magnitude can have significance, namely 0.0001 mgm. oxygen per gram of *Valonia* per hour. With a respiratory quotient of unity, the thin disks of potato tissue used in salt absorption experiments would have an oxygen uptake of about 0.15 mgm. per gram per hour, or over 1000 times the rate of *Valonia.* By direct measurement Ulrich[1] has shown that barley root systems active in salt accumulation have oxygen uptake of the order of 0.30 mgm. per gram per hour. This difference need not cause surprise if the small amount of protoplasm in *Valonia* is recalled[2] and also the fact that, although its manner of growth is obscure, these large vesicles are analogous rather to quiescent mature parenchyma cells than to those which are still, or have again become, active in growth and metabolism. The very low oxygen uptake suggests the explanation for the fact that *V. ventricosa* will survive long periods in darkness in a relatively small volume of clean sea water.

The general level of respiration of these large vesicles is then to be compared rather with that of red blood cells than with cells of higher plants active in metabolism and salt accumulation. This disparity in metabolic activity between *Valonia* and other more typical systems which freely accumulate both anion and cation from dilute solutions cannot be ignored. Hence, *Valonia* should be regarded as a special case physiologically, as it certainly is morphologically, and, though of interest for its own sake, it is hardly an organism from which generalizations should be derived and freely extended to all plants. One of us (F. C. S.) has attempted to illustrate these general relationships (80), and in the light of this more extended treatment of the results obtained at Tortugas, there is no reason to depart from the principal views then expressed.

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[1] Experiments in the Division of Plant Nutrition, University of California.

[2] The comparison really required is the amount of metabolism per unit volume of water in which the accumulation occurs. For all practical purposes this is given by the comparison of rates of metabolism per unit of fresh weight.

SUMMARY OF SECTION V

Valonia will not accumulate bromide from sea water enriched with potassium bromide.

Experiments in the light, in the dark, and in the dark with the solutions adjusted to cylinder nitrogen showed that these treatments did not affect the concentration of the sap constituents of *V. ventricosa.* These results suggest differences between *Valonia* and other plants.

The carbon dioxide output of *Valonia* in the dark is insignificant.

The oxygen production by *V. ventricosa* in the light has been demonstrated. The absorption of oxygen in the dark is real but of small magnitude.

The respiration rate calculated on the basis of oxygen uptake is of a very much lower order of magnitude than that of plants active in the accumulation of anions and cations from dilute solution.

GENERAL COMMENTS

Section V shows that the outstanding metabolic property of the large vesicles of *Valonia* is their comparative inactivity, with which must also be associated the fact, commonly overlooked, that their ability to accumulate salts is relatively small. The high concentration of chloride in the sea permits *Valonia* to acquire the total salt concentrations adequate for growth with but a very small degree of accumulation (accumulation ratio less than 1.2) of this the only anion present in the sap in quantity. Indeed it is doubtful whether *Valonia* possesses the ability to accumulate any other anion.[1] Potassium is the only cation which exists in the sap in a concentration higher than in the sea, and this might be largely at the expense of sodium, by a process analogous to the replacement of chloride by bromide in *Nitella* (34), a process which, though not independent of metabolism, does not demand the same degree of activity as the simultaneous uptake of anion and cation from dilute solutions. The difference in osmotic pressure between sap and sea water is also small in comparison with the differences frequently encountered in cells which exist in contact with dilute solution. Although the wall of the vesicle seems to be adapted to resist great pressures, the wall pressures it actually exerts when the vesicle exists in osmotic equilibrium with sea water are trifling relative to those necessary in the majority of plant cells which will tolerate very dilute external solutions. Despite the fact that enlargement has been stated to be dependent upon synthesis of cellulose (44), it seems that the real limitation to salt concentration in *Valonia* must be inherent in its comparatively slow metabolism. The results of the experiments of section IV clearly indicate that the increment of internal concentration is conditional upon an increment of external salt concentration which then permits the system to readjust to a new condition of balance—a result which is analogous to Collander's findings that in water of varying salinity the salt content of *Chara* varies greatly but the osmotic pressure difference between sap and medium is constant (20).

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[1] Both Wodehouse (87) and Osterhout (54) state, on the basis of color tests, that nitrate accumulates from the sea though the amounts present were very small. Ullrich (86*a*) made permeability measurements on *V. macrophysa* using the anions nitrate, bromide, and salicylate, but accumulation of these ions was not involved.

The conspicuous problem presented by *Valonia* resolves itself into the mechanism which determines the great disparity in the absorption of one cation (K) over another (Na). The more general problem of salt accumulation in plants is that presented by systems (30–35, 69, 74–84) able to absorb both anion and cation, in approximately equivalent amounts, and thus transfer salt from very dilute to relatively strong solutions. Most fresh-water algæ and parenchyma cells of flowering plants will accumulate a variety of both anions and cations until high accumulation ratios are obtained. It is the study of the latter systems which leads inevitably to the conclusion that their ability to do this resides in their capacity for active metabolism. The necessity for a high metabolic rate is first to maintain the organization capable of active salt accumulation, but it may also involve the direct utilization of energy to produce and maintain the observed concentration relations between vacuole and external solution. The mere production of carbon dioxide or the maintenance of specified pH relations between sap and external solution will not alone suffice (82, 30). This aspect of the problem has been suppressed in the work on *Valonia*, principally because these organisms possess the properties of *salt* accumulation and metabolism in but slight degree. It is possible so to concentrate on the disparity between potassium and sodium in the sap that the chloride concentration is treated as of secondary importance. This has certainly occurred in the theoretical speculations concerned with the "gradients of thermodynamic potential of free base" and also with the models. This is evident from the fact that in the writings on *Valonia* the references to simultaneous accumulation of potassium and chloride are confined to fragmentary statements that of course this requires energy. In the latest review by Osterhout (59) the section headed "Thermodynamics—expenditure of energy" consists of three short sentences which conclude with the phrase "a general accumulation requires energy." The most recent models (66) do not demonstrate the simultaneous accumulation of potassium and chloride, in fact in the phase representing the vacuole the chloride concentration actually declined from equality with the external solution as the experiment progressed. The mere fact that the value of the product K×Cl in the internal phase increased over that in the external cannot compensate for this shortcoming. The results of section IV indicate that though the accumulation ratios of chloride may not be impressive, this is the most important ion in regulating the concentrations in the sap. It is quite evident that the clue to the mechanism of the accumulation of *salt* lies with the rôle of metabolism. No amount of work on non-living models can attain this end, as they are based on the illusion that it is the mere presence of carbon dioxide which regulates accumulation. All the detailed evidence from simultaneous studies of salt accumulation and metabolism (30, 70, 77, 80, 81) shows that it is not the carbon dioxide produced so much as the oxidative reactions which precede this that are causally concerned with salt accumulation. The causal connection between respiration and salt uptake is not a simple and direct one.

Valonia then presents special opportunities for the study of those factors which cause the great disparity in the penetration of ions even though similar in chemical nature (e.g. K and Na)[1] or the replacement (exchange) of potassium by other cations. This is the aspect of salt absorption previously designated "induced" rather than "primary" in character (80). For the general problems of salt accumulation the utility of *Valonia* is severely limited by its very restricted capacity for the accumulation of different ions and the very low degree of anion accumulation which it can produce. Granted the ability to accumulate salts, or salts accumulated during an earlier more active period of metabolism, the factors which govern the disparity in the uptake of one ion over another, or the mere exchange of an ion with another in the sap, must surely be explicable in terms of the known properties of the ions concerned. The inherent capacity to accumulate salt, however, resides in the living system, and it is here that the emphasis must be; rather upon the processes of growth and metabolism than upon the mere physical properties of ions or molecules or their concentration relations. The more active the system examined, the more evident does this become. In view of the difficulties it presents, it is improbable that further work on *Valonia* will provide that knowledge of those biochemical processes which link the metabolism and the process of *salt* (anion and cation) accumulation. The unique morphology of the *Valonia* vesicle presented an exceptional opportunity to decide whether the accumulated salts in plants could all exist in true solution. Although it is often stated that a sap analysis can be made from a single vesicle, the practice is rarely resorted to. Analyses of sap from single vesicles would be accompanied by even greater variability than that already described, and the advantage of this in experiments seems difficult to appreciate. In short, the very features which first attracted attention to these organisms now constitute their chief limitations.

[1] This aspect of the study is still capable of much extension. Despite the recent works of Brooks (15) on rubidium and earlier references to the uptake of other alkali metals (*loc. cit.*, citations), a complete study of the relations which determine the penetration of the different alkali metals, for which *Valonia* is well adapted, still remains to be made. Such an investigation is now in progress at Tortugas.

Much may still be done by the further examination of the factors which determine the sap composition of these plants in their habitat, especially if the differences observed can be correlated with environmental factors and their metabolic effects determined. As yet neither the factors which operate in situ nor those which affect the sap composition of isolated vesicles under experimental conditions at Tortugas support the theory of Osterhout, which attaches an exaggerated importance to the pH relations of the sap and sea water.

The ultimate interpretation of those biological phenomena which are inseparably dependent upon the organization of the living system involves peculiar difficulty, which is intensified when the process in question must be identified with such intimate properties as those of growth and metabolism. The trend of recent evidence shows that the property of salt accumulation is of this kind. Until the detailed biochemical processes involved in salt accumulation can be described, mechanistic speculation seems to be superfluous. Relative to the special case of *Valonia*, which is distinguished by the fact that, of all the plants extensively studied from this point of view, the metabolism is the most intangible and the responses of the sap concentration to external variables the least impressive, speculation and copious reference to non-living models has aroused the hope that the general problem of salt accumulation in plants can now be regarded and described in terms of known processes in vitro. Any such hope is premature and is the result of a misplaced emphasis upon principles derived from an organism the special features and limitations of which do not seem to have received in the past the attention they deserve.

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APPENDIX

THE ANALYSIS OF VALONIA SAP

Simple though the sap of *Valonia* is in composition, its quantitative analysis presents difficulties which should not be ignored.

There are two problems to be considered: first, the reproducibility of any given procedure under the conditions in which it is employed, and, second, the absolute values it yields. The first consideration is perhaps the more important in any given series of experiments and is adequately evaluated by replicate determinations under standardized conditions. The derivation of results which might be termed "comparable" within the group by a given method caused little embarrassment in this work. It is when absolute comparisons between the different sap constituents are made that difficulties are encountered.

The ultimate justification for the procedures finally adopted must rest not merely upon the evidence of their applicability to *Valonia* sap, but rather upon the confidence in them gained from experience of their application to the analysis of plant and soil extracts in the Division of Plant Nutrition, University of California.

In order to evaluate the progress of the work it was necessary that analyses should be made at Tortugas. This necessitated analytical work under conditions which tend to aggravate the ordinary manipulative difficulties. Experience showed that these difficulties can be surmounted. The methods employed were necessarily selected with due regard to speed and simplicity. However, considerable importance must be attached to the fact that, in nearly every case, replicate analyses have been made using different methods, and frequently parallel determinations were done by different individuals. It is quite evident that the major conclusions can be derived independently of the slight vagaries peculiar to any method or of personal factors in its application.

Chloride has been determined in several ways. Some early experiments utilized the familiar NH_4CNS–$AgNO_3$ method with an iron alum indicator. The use of alkaline wet ashing to remove traces of organic matter, a procedure commonly desirable, was quickly abandoned as superfluous. Precision, however, still demanded that the precipitated silver halide should be filtered and washed prior to the titration of excess $AgNO_3$ with NH_4CNS. The accuracy of this technique is unquestionable, but it is rather laborious for use with a large number of samples.

Direct determination of chloride is possible in two ways. The first, using potassium chromate as indicator, is too familiar to require citation. In the second, the titration is carried out in the presence of acetone and dichlorfluorescein as indicator (46). Both procedures have been used and they yield the same result. Twenty-five samples were analyzed by both methods. Nine results were identical; in fourteen cases the dichlorfluorescein method yielded slightly higher results. The average difference between the two methods applied to *Valonia* sap is given by the formula

$$\mathrm{Cl}_{\mathrm{dichlorfluorescein}} - \mathrm{Cl}_{\mathrm{chromate}} = 0.002.$$

For all practical purposes this difference is negligible. Most of the chloride data in this paper were obtained by the chromate method.

There is the further problem presented by the chloride determination carried out on freshly extracted sap and the same determination on preserved samples. Sterilized, liquid sap samples may be preserved in sealed vials (13). In 1933 the procedure preferred was to measure into a clean, dry vial, drawn ready to seal, a known volume of sap. This was slowly evaporated in an oven and eventually sealed off when dry. Finally the entire contents were washed out quantitatively. Both these procedures were cumbersome, and for most purposes the extracted saps were satisfactorily stored under toluene in stout, small, narrow-mouthed vials which were thoroughly waxed after the sap was inserted.

All the samples included in this paper were analyzed for chloride at Berkeley, California. Twenty-five samples analyzed both at Berkeley and at Tortugas showed that the Berkeley figures were slightly higher. The mean correction was 1.3 per cent, and this includes all errors inherent in the use of different volumetric apparatus or standard solutions, or due to the different temperature conditions. The Berkeley figures are preferred except in a few cases (15 in all) where there is reason to believe that a real difference was introduced between the first analyses at Tortugas and the subsequent analyses at Berkeley. These samples were completely analyzed at Tortugas, and it was not at first intended to repeat the determinations, so that they were not immediately stored and sealed in a form suitable for transit. In these cases the true chloride concentration was derived from the Tortugas figures, to which the above-mentioned correction was added. This chloride figure then served as a standard of reference from which the subsequent potassium and sodium analyses done at Berkeley could be recalculated. There is no reason to doubt the figures thus obtained.

The most difficult feature of the analysis of *Valonia* sap is the determination of potassium and sodium. For rapid use at Tortugas the volumetric cobaltinitrite method under the conditions prescribed by Hibbard and Stout (29) was adopted. This method was used after preliminary ignition with concentrated sulphuric acid. The reproducibility of the results obtained may be appreciated from the fact that from 14 samples determined simultaneously and in duplicate the average deviation from the mean was only 0.33 per cent. Thirteen of these samples, reanalyzed at Berkeley by the same method, yielded figures which in no case deviated from the Tortugas mean by 2×0.33 per cent. The method using the sulphuric acid ignition, therefore, gave the same results at Tortugas and at Berkeley. It was found, however, that the volumetric cobaltinitrite method applied without ignition with sulphuric acid gave slightly higher results on the same solutions (of 13 cases only 1 gave a lower result). The order of this difference is given by the average deviation from the figures obtained with sulphuric acid ignition, namely 2.7 per cent. This aroused some uncertainty concerning the best figures for absolute, rather than relative, comparison and prompted the further use of the more laborious gravimetric method, in which potassium is determined as K_2PtCl_6. Long experience has shown that this method is more consistently reliable than any other, not excluding the volumetric cobaltinitrite method of Hibbard and Stout.

The use of the chloroplatinate method is, however, not entirely free from the difficulty that the results differ slightly if the method is used with and without sulphuric acid ignition. Invariably the results are slightly lower if the ignition is employed. Nevertheless the greatest reliance has been placed upon the potassium determinations derived by this means, and in the event that a choice was made between the results of parallel determinations by different methods, this was the one chosen. This necessitated the repetition at Berkeley of almost all the analyses done at Tortugas. All the potassium figures, therefore, to which special significance was attached were the results of analyses by the chloroplatinate method or were appropriately corrected to this basis. A contributory factor in this choice was that the determination of potassium could be combined with that of potassium plus sodium as sulphates and the sodium was then derived by difference. The sum of the potassium and sodium concentrations thus derived agreed most closely and consistently with the chloride figures obtained as described. The balance of potassium plus sodium against chloride is a useful check on the accuracy of the results of analyses of *Valonia* sap. Wherever there was an appreciable discrepancy the analyses were repeated, and if the difference was not eliminated the figures were regarded as open to question.

The distribution curve for potassium content of *V. macrophysa* (fig. 3) was plotted upon data obtained by the volumetric method after sulphuric acid ignition at Tortugas. From 59 cases where saps were analyzed by this method and also by the gravimetric method (with ignition) it was calculated that the mean derived should be reduced by 0.0033 gm. equivs. per liter to correct it to the chloroplatinate basis. This correction, with its errors, was incorporated in the calculations from which table 4 was derived.

The determination of sodium was tried by three methods, namely, by the uranium method as described by Barber and Kolthoff (3), and by weighing either the combined chlorides or the sulphates of potassium and sodium and then deducting the potassium value obtained as above. All the methods were in close agreement, with due regard to the difficulty of the determination of sodium in the presence of a large excess of potassium. For reasons stated in the text, small, experimentally induced changes of sodium content have no significance because the variability of the plants is so great relative to sodium content.

The only important use made of the sodium figures was in the treatment of the sap as it occurred naturally. A random selection from a number of cases in which the sap was analyzed by the three methods shows that they are in close agreement (table 27). For reasons already stated, preference was finally given to the determinations as combined chloride or sulphate. The chief utility of the direct determination of sodium was that, since it yielded values so close to those obtained by the indirect method which also involves the determination of potassium, it strengthened the confidence already placed in this procedure, and further the comparison of the combined potassium plus sodium with chloride was a valuable guide to the elimination of the scanty but occasional errors which arose in these determinations.

TABLE 27—*Representative analyses of sap of Valonia ventricosa: sodium determinations by three methods*

Direct determination as sodium uranyl acetate	Indirect determination: K and Na weighed as	
	Sulphates	Chlorides
0.032	0.034	0.030
0.024	0.020	0.021
0.034	0.028	0.021
0.027	0.020	0.024
0.043	0.050	0.055
0.041	0.048	0.048
0.046	0.031	0.034
0.047	0.039	0.037
0.038	0.031	0.031
0.060	0.053	0.055

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PAPERS FROM TORTUGAS LABORATORY, VOL. XXXI

VII

THE MECHANICAL SIGNIFICANCE OF THE POSITION AND MOVEMENTS OF THE PAIRED FINS IN THE TELEOSTEI

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With eight text-figures

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THE MECHANICAL SIGNIFICANCE OF THE POSITION AND MOVEMENTS OF THE PAIRED FINS IN THE TELEOSTEI

INTRODUCTION

In the course of the evolution of the modern teleostean fish, a series of fairly well-defined changes has taken place in the body form and in the shape and position of the fins. It has been the endeavor of the writer to correlate these changes with the mechanical factors concerned in fish locomotion. This paper is an attempt to treat the problem in a general fashion, without going too deeply into the mathematical side of the mechanics; a treatment which will be the more satisfactory since our knowledge of the hydrodynamic properties of moving bodies is insufficient to handle this problem more than qualitatively.

We may illustrate the principal changes involved in this evolution by a consideration of the main differences between a typical shark and a teleost from the family Percidæ. It must be admitted at the outset that we know of no connecting link between the two types. It seems reasonable to suppose that the extinct Palæoniscoidei are ancestral to the modern Teleostei, but the link between the palæoniscids and the cartilaginous group of fishes has yet to be discovered. Palæontology has up to now given us no better alternative than to treat the evolution of the fishes as a number of disconnected lines of development. One line represents a somewhat heterogeneous collection of "cartilaginous" forms, whose interrelationships are obscure. A second line starts with the Palæoniscoidei and through the successive stages of Chondrostei and Holostei terminates at the present day in the highly specialized Teleostei. A third includes the fossil Crossopterygii, which link up with the Dipnoi, and are evidently allied to the tetrapod stem from which are derived all the land-living vertebrates.

From the brief statement above it may easily be seen that an attempt to define the evolution of the teleost fish form in terms of a comparison between a shark and a percid is not really satisfactory, but it is still true that if we can strip from the shark all of its undoubted specializations, and concentrate upon the principal features of its mechanical organization, we shall arrive at a type which could be logically regarded as ancestral to both the Teleostei and Palæoniscoidei. The features which by the writer are considered to be primitive in the form of the shark are the following:

1. The presence of a heterocercal tail, asymmetrical both internally and externally.
2. The absence of an air bladder.

3. The possession of pectoral fins which are large in area, placed low down toward the ventral side of the body, and having their axis approximately horizontal. The form of the fins tends to the pleurorachic type, with a broad base. In consequence of this broad base, the amount of rotation which the fin can perform about a horizontal axis (transverse to the main axis of the fish) is very limited.

4. The presence of small pelvic fins, which have a horizontal axis and a broad base, and are situated well behind the center of gravity of the fish.

In contrast to these features, the advanced characteristics of the percid fish are:

1. The tail is homocercal, which implies that though showing a morphological internal asymmetry, it is externally and mechanically symmetrical in form.

2. There is typically an air bladder, though in a few specialized forms this may be lost.

3. The pectoral fins are generally much smaller than those of the shark. They are inserted relatively high up toward the dorsal side of the body, and their axis is rotated into a position which approaches the vertical. The base of the fin, however, is narrow, so that a considerable freedom of rotational movement is attained.

4. The pelvic fins tend to move forward to such an extent that they may even be anterior to the pectorals. The pelvic fin axis is still more or less horizontal in direction, but there is a much greater power of movement than in the pelvic fin of the shark.

These changes in general body form are associated with a number of others of rather less importance. The shark type is relatively long and thin, and the cross-section of the anterior part of the body indicates a definite flattening of the ventral surface. Furthermore, the fins in the teleost are actinopterygial. Morphologically, this means that their basal and radial elements have sunk below the level of the body surface, so that the fin rays alone are left projecting, connected together by a thin fin membrane. Mechanically, it implies that the fin is collapsible, capable of changing its effective area, and that its movements are effected by muscles which lie beneath the body surface, since they are inserted on the bases of the fin rays. The position of the mouth in sharks is typically subterminal, on the ventral surface, while it is terminal in most teleosts. Finally, the median fins of the Teleostei tend to be divided into two clearly defined regions: an anterior erectile portion, capable of only very little lateral inclination, and a posterior flexible portion in which the extent of the lateral motion is very great, and the erectile nature of the fin rays much less strongly marked.

It is believed that all these differences are concerned mainly with mechanical changes in the two types; differences which, when they are correctly interpreted, give a reasonably clear picture of the evolutionary changes which all such forms must pass through in a quest for greater efficiency of the locomotor mechanism. The equilibrium of the two types is considered separately in the following paragraphs.

THE EQUILIBRIUM OF THE SHARK

That the heterocercal tail in its motion exerts an upward force has long been known. Ahlborn (1896) pointed out the reason for the existence of this vertical force, and the most recent work of Grove and Newell (1936) has confirmed his deductions by the use of a series of mechanical models. The force arises by virtue of the fact that in the lateral motion of the tail during swimming, the large hypocaudal fin lags behind the motion of the rest of the caudal fin. The consequent inclination of this lobe to the vertical produces a force which has upward, backward, and lateral components. In the two directions of motion of the caudal region, the lateral components of this force are equal and opposite; they therefore cancel out. But the backward and upward force components are similar in direction and magnitude in both halves of the stroke, owing to the changed angle of attack of the tail fin to the water. The backward component contributes to the swimming force, while the upward component is an essential feature of the swimming equilibrium.

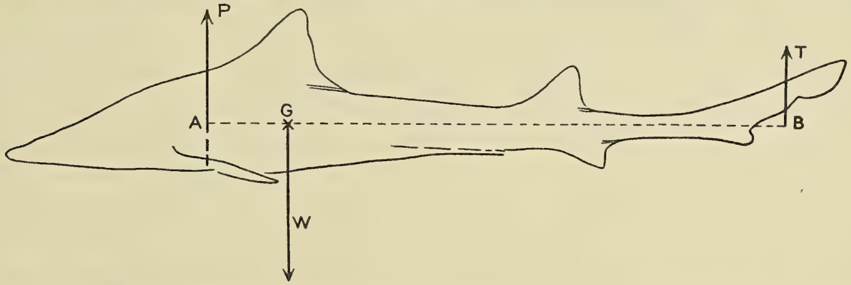

FIG. 1—The equilibrium of the dogfish, *Mustelus canis*, during swimming. The vertical components only of the forces are shown. The pectoral fins produce an upward force, P, acting through the point *A* on the horizontal axis of the fish, while the vertical component, T, of the tail force acts through *B*. The moments of these two forces about the center of gravity, *G*, must be equal and opposite; i.e., $\mathbf{P}.\overline{AG}=\mathbf{T}.\overline{GB}$. The weight of the fish (immersed in water) is W, acting at *G*. It follows, therefore, that P+T=W.

The presence of this constant source of lift at the posterior part of the body produces a tendency for the fish to somersault in a forward direction, forcing the head downward and the tail upward. It is compensated in the dogfish by an equal and opposite effect produced by the trailing pectoral fins. This action of the pectoral fins has been shown by the writer (Harris, 1936) in a series of wind-tunnel tests on an accurate model of *Mustelus canis*. These fins also produce a lift force, but as they are situated anterior to the center of gravity, the "somersaulting effect" is in an upward and backward direction, tending to raise the head. In normal swimming, this effect exactly neutralizes that of the heterocercal tail, so that the body remains horizontal while swimming. The diagram (fig. 1) illustrates the balance of these forces. It will be seen that while the pitching moments (i.e. the "somersaulting tendencies") are balanced, as indicated by the equation

$$P.\overline{AG}=T.\overline{GB},$$

there is a total resultant lift force thereby produced, equivalent to an upward lift of P+T through the center of gravity. This force is balanced by the effective weight of the body W. In these shark types, the specific gravity is almost invariably greater than that of the surrounding medium, so that there is a tendency for the fish to sink toward the bottom. This always happens when the fish stops swimming, but during active swimming movements the combined resultant lift of the pectoral fins and of the tail serves to keep the fish on a horizontal and level course. Magnan (1929) gives a number of data for the relative densities (compared with sea water) of a number of sharks. The mean of his values is 1.034, so that in a fish of 1 kilogram weight (in air) the total lift force required to maintain a level course is a little over 30 grams.

In order to produce a relatively large vertical lift force, and little drag, the pectoral fins of the shark are large in area, and are trailed at a very small angle of attack. According to the observations of the writer, the angle of incidence of the pectoral fin plane to the body axis in the dogfish is rarely more than 8°, and is usually less than this. In this respect the fins behave in a very similar manner to the aerofoil (wing) of an aeroplane, which also produces a large lift at the expense of a relatively small drag force. A very slight change in the angle of incidence of the fins will produce large upward or downward forces, and vertical control is thus achieved with little effort. This comparatively effortless control is further assisted by the fact that the position of the large pectoral fins anterior to the center of gravity makes the fish body statically unstable (in the aeronautical sense of the word), and the controllability is therefore high. (For a more detailed explanation of this subject of "stability" and "controllability" the reader is referred to the paper previously mentioned, Harris, 1936.) The large horizontally directed fin base which is the keystone of this mechanical system is certainly a primitive characteristic, since we have every reason to believe that the primary lateral fin folds of the fish were inclined at a small angle to the horizontal axis of the fish body.

The wide base of the pectoral fins is appropriate to a form in which the pectoral musculature is relatively weak, and the angle of incidence has to be held fairly constant; this, however, is also an undoubtedly primitive characteristic of the fish fin.

It seems probable that the extremely low position of the pectoral fin base in many sharks may be to some extent a secondary feature. The pectoral fins in these fish are used partly to prevent rolling, as keels are used in shipping. In order to exert a maximum anti-rolling effect, the position of a keel should be adjusted so that its distance from the center of roll is as large as possible. In view of the ventrally flattened shape of these sharks, it is seen that the three "corners" of the triangular cross-section are the obvious positions for such keels. The dorsal fin is inserted along the top edge, and the pectorals at the lower corners (see Bryan, 1900).

THE EQUILIBRIUM OF THE TELEOST

Since the heterocercal tail is a mechanism for the lifting of the posterior end of the body, its disappearance is presaged immediately the evolution of the air bladder eliminates the necessity for this lift force. Strong evidence for the truth of this assertion can be derived from the fact that in each of three lines of evolution of the fish, the development of an air bladder is followed by a gradual change in form of the tail from the asymmetrical to the externally symmetrical type. This change has occurred independently in the palæoniscoid-teleost evolution, in the Dipnoi, and in the Crossopterygii, but in each of the three cases a different method is used to bring about the external symmetry (see Graham Smith, 1936). In the teleost evolution, the change to the symmetrical form involved a very considerable modification of the tail structure, and the process took a much longer time than in the other two groups. In the Dipnoi and Crossopterygii, enlargement of the dorsal fin areas and (in the former) fusion with the enlarged epicaudal lobe of the tail brought about a secondary symmetry which was accompanied by a natural bending of the vertebral axis of the tail back to its horizontal axial position. In the Teleostei, on the other hand, the shortening of the upturned axis of the primitive heterocercal tail fin progressed to such an extent that the fin rays finally came to be directed backward, giving once more a symmetrical thrust to the water; in this case, however, the internal structure of the tail shows that it is still morphologically asymmetrical.

Since the tail lift was no longer present in these forms with a symmetrical caudal fin, there was no longer any pitching moment produced at the posterior end of the body. The pectoral fins were therefore released from the necessity of providing a balancing force for the tail, and (also by virtue of the presence of an air bladder) from the necessity of producing a lift force to neutralize the weight of the fish. Provided such a symmetrical tail were combined with a body which was very flexible in a vertical plane, it would apparently be possible to dispense completely with the paired fins, relying on flexure of the body to produce rising and diving movements. This is probably the explanation of the existence of many eel-like forms, where the pectoral fins are so far reduced that they no longer contribute to the equilibrium of the fish. But it is equally possible that the release of the pectoral fins from the necessity of producing a continuous lift force would permit of the much more typical development of these structures as brakes and steering organs, their characteristic rôle in the Teleostei. In certain fish, they are also developed as powerful locomotor organs.

If the pectoral fins are to develop a new function as brakes, it is obvious that in order not to produce a somersaulting force, their resultant must be made to act through the center of gravity of the fish. For if a powerful drag force is applied by a fin situated far below the axis of the body, it will tend to push the nose of the fish downward, and this effect would need to be compensated by some other force. This is actually found in many primitive groups of the Actinopterygii, as in the holostean *Lepidosteus,* and also among the Haplomi (e.g. *Esox*), where the pectoral fins are placed low down on the side of the body. The braking action of these fins produces a pitching moment which is compensated by the action of the pelvic fins. Breder (1926) has already pointed this out, but it seems likely that his diagram (fig. 57B, p. 206) of the action of the pelvic fins during a sudden stop must be erroneous, since an additional drag on these fins would only increase the somersaulting tendency. It is more probable that the force exerted by the pelvic fins is backward and *downward,* so that the tendency for the tail to rise is opposed by the downward thrust of the pelvic fins, as in his figure 57A.

In order to develop a powerful drag force, with relatively little lift force, the axis of the teleost pectoral fin rotates toward a vertical position. At the same time, in order to make this resultant force pass through the center of gravity, the position of the insertion of the pectoral fin base rises toward the level of the body axis. This process is illustrated in figure 2. The axis of the fin does not rotate completely into the vertical position, since such an arrangement is not suited to the muscular anatomy of the fin. Powerful erector muscles run from the cleithrum and scapula to the anterior fin ray, and the consequent strengthening of the upper border of the fin makes it serve best as a strongly braced leading edge for a fin which is inclined steeply, but not quite vertically. These erector muscles play a most important part in the abduction of the pectoral fin as a brake (e.g. in the Scombridæ), and the fin axis is therefore inclined to the vertical so as to allow them to abduct and erect the fin in this inclined position. Such an inclined plane will exert a lift component as well as a drag force, and it is common to find that the pectoral fins in Teleostei are situated rather below the level of the center of gravity of the fish, so that the resultant of this lift and drag (**R**, fig. 2B) passes through the center of gravity. In this way there is no couple tending to tilt the head of the fish upward or downward during a stop.

Since, however, the fin is inclined to the vertical, though the resultant pitching moment may be zero, there remains a lift component, small in magnitude, but enough (as experiments by the writer on the sunfish, *Lepomis auritus*, have shown) to make the fish rise in the water when it attempts to stop. In order to compensate for this lift force, and to ensure that the fish continues on a horizontal path during its deceleration, a downward force must be introduced. It is not sufficient to make the fish body a little heavier

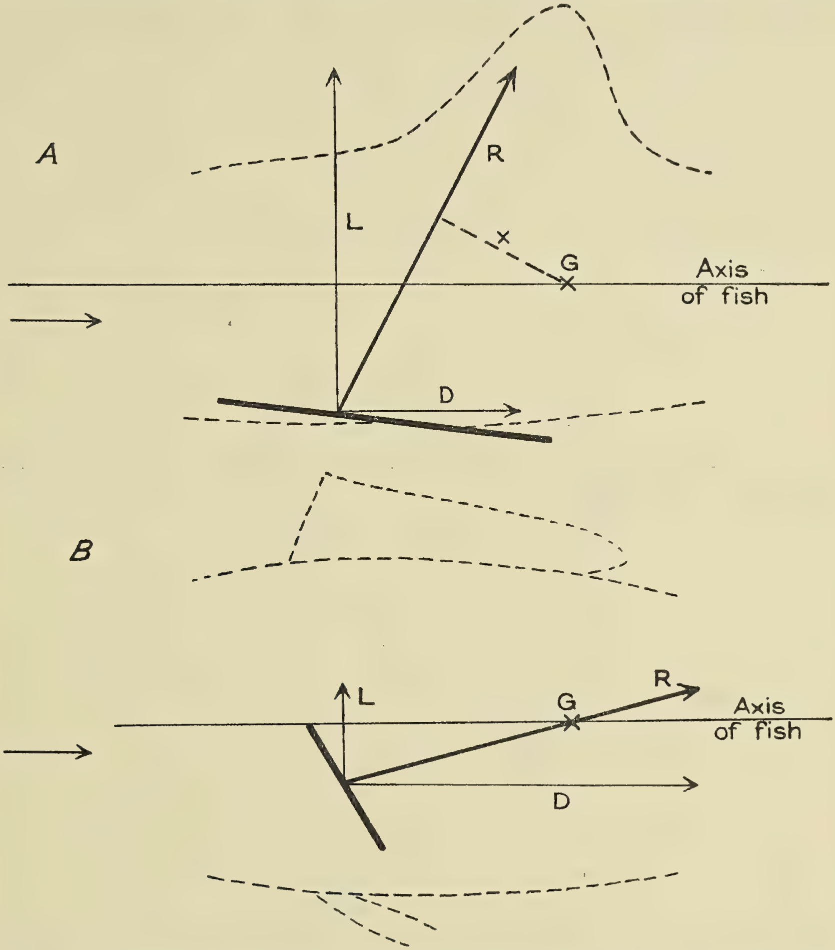

Fig. 2—Illustrating the forces exerted by the pectoral fins in *A*, a shark, and *B*, a percoid teleost. The fins are assumed to be stationary. The small arrows on the left indicate the direction of the water flow against the fins. The dotted lines represent the outline of the fish body in the pectoral region; the heavy line, the section of the fin plane; **R**, **L**, and **D**, the resultant force, and its lift and drag components on the fin. In diagram A, x is the length of the perpendicular from the center of gravity, *G*, to the resultant **R**. The positive pitching moment in A is therefore $\mathbf{R}x$, and in B is zero, if **R** passes through *G*.

than the water by suitable adjustment of the size of the air bladder, since this system is not responsive to rapid changes in specific gravity, and since the lift force on the pectoral fin varies with the speed of the fish, and is therefore changing throughout the process of a stop. The compensating force is therefore introduced by another fin system—the pelvics. A downward force produced by these fins will vary with change in the fish velocity in just the same manner as does the lift produced by the pectoral fins, and the equilibrium will be constant throughout all the changes in speed.

The only difficulty in using the pelvic fins to produce this downward force is that the new force thus introduced, has again a pitching moment about the center of gravity, and if the pelvics are placed far back along the body, this downward force tends to raise the head. It can readily be shown that if the pectorals are to produce the maximum possible braking effect, the pelvic fins must be moved forward to a considerable extent. Figure 3 illustrates in a diagram the force equilibrium of the pectoral and pelvic fins of a typical percoid. See also Harris (1937).

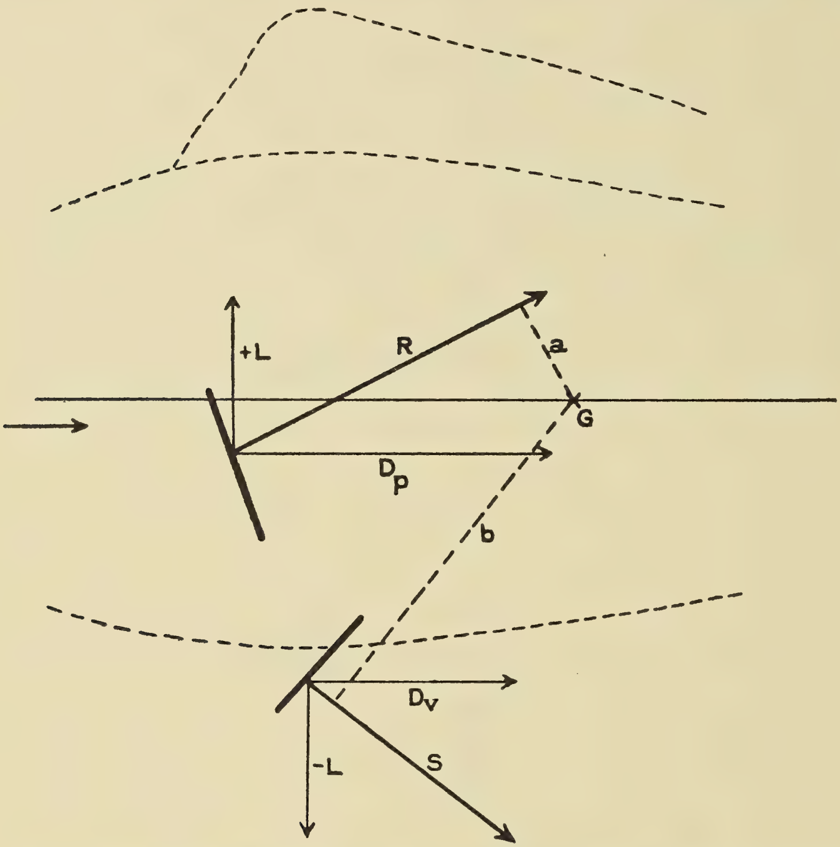

FIG. 3—The equilibrium of forces in a typical percoid during the joint action as brakes of the pectoral and pelvic fins. As in figure 2, heavy lines represent the equivalent sections of the fin planes; dotted lines the outline of the body in the pectoral region; **R**, **S**, the resultant forces acting respectively on the pectoral and pelvic fin. **R** has a lift component, **L**, and a drag component, $\mathbf{D}_\mathrm{P}$; **S** has a vertical component, **L** (which must be equal and opposite to the lift of the pectoral fin), and a drag component $\mathbf{D}_\mathrm{V}$. *a* and *b* are the perpendiculars from the center of gravity *G* to the two resultants **R** and **S**. For pitching equilibrium, $\mathbf{R}a = \mathbf{S}b$. There is no resultant vertical force, since the lift components are in equilibrium, and the horizontal force, $\mathbf{D}_\mathrm{P}+\mathbf{D}_\mathrm{V}$, is the total braking force exerted by the fin system.

FIN MOVEMENTS IN THE TELEOST

Up to the present we have considered the mechanical factors concerned in the evolution of the fin pattern by treating the fins as plane surfaces, inclined at a definite angle to the body surface. But it is obvious that this is only a part of the evolutionary story of the teleost fin. One of the greatest advances in the phylogeny of the actinopterygial fin is its increased power of independent motion. The musculature of the shark fin seems to consist essentially of a protractor set of fibers and a retractor set. Part of each series acts on the dorsal side of the fin (elevators) and part acts on the ventral side (depressors). The distinction between the muscle groups is nowhere very clear, and the series from the anterior to the posterior border of the fin is innervated by a regular succession of segmental nerves, as shown by Brazier Howell (1932). In contrast to the selachian type, the percoid fin shows a great differentiation into functional muscles. The segmental nature of the fin ray muscle bundles is still completely preserved (unpublished work of the writer), but the grouping of the muscle fibers into no less than eight distinct muscles permits a vastly more complicated response than is possible in the shark fin. It is interesting, nevertheless, to find that even in these complicated motions, the regular succession of motor impulses along each segment in turn, characteristic of the body myotome contractions during the normal swimming of the eel (see e.g. Gray, 1933), is still observed in the fin; there is a similar phase difference between the beats of the successive fin rays. During forward locomotion produced by pectoral fin movement the wave of contraction of the muscles passes from top to bottom of the percoid fin—in other words, from the morphological anterior border to the posterior—while during backing movements the wave usually passes up the fin in the reverse direction. This direction of passage of the motor impulses thus corresponds exactly to the direction of passage of the myotome contractions used in normal forward or backward body swimming, suggesting that the whole locomotor system is worked on a similar basic pattern.

The characteristics of the undulatory movement thus produced in the pectoral and median fins of the fish vary greatly in different fish species. In many Plectognathi (e.g. in *Spheroides*), the phase difference between successive fin rays is considerable, so that the edge of the fin is bent into a curve which may include as much as three-quarters of a complete wave form. In other Plectognathi (*Balistes*), the phase difference is much less, and in percoids such as the serranid *Epinephelus*, cinematographic studies have shown that the total difference in phase between the top and bottom of the fin is only 60° (i.e., one-sixth of a wave form). In the pectoral fins of the Scombridæ, where the fin base is much less flexible, it is certain that the phase difference between the upper and lower borders of the fin during the abductor movements must be even less than this.

Where the phase difference between the beats of the successive rays is large, the visible effect is the production by the fin of a characteristic undulatory form when moving; where it is small, the fin seems to move almost as a plane surface. The difference is illustrated in diagrammatic form in figure 4, which shows the beat of two such fin types seen from the side.

If, as in the diagram of figure 4, the beat of each fin ray is symmetrical about a mean position which is perpendicular to the side of the fish body, it will be obvious that the longitudinal force produced when the fin moves forward (abduction) will be equal and opposite to that produced during the other (adduction) half of its beat. There will therefore be no tendency to propel the fish in either a forward or a backward direction. But the passage of such a wave down the fin obviously produces a downward-directed current of water, and therefore an upward force on the fish, which can be used to produce either vertical motion (translation) or pitching and rolling effects (rotations). This method of production of vertical forces by the use of the pectoral fins is widely used in the Plectognathi, and is found in almost all the teleostean groups. In *Lebistes* (Haplomi), when combined with a vertical undulation of the tail fin, such a pectoral fin motion can make the fish rise vertically in the water like a helicopter. In the Plectognathi, this motion is largely used to control the pitching equilibrium of the fish, an undulation traveling down the fin tending to raise the head, while a reverse one depresses the head end.

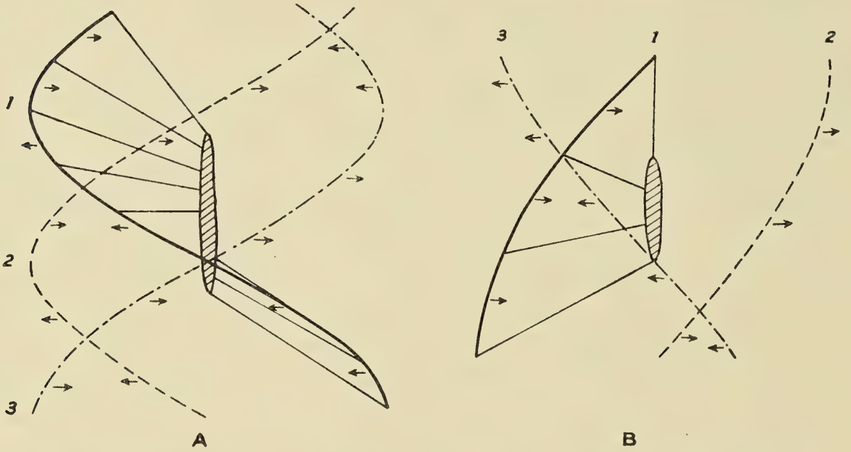

FIG. 4—Diagrammatic representation of the motion (seen from the side) of a pectoral fin, undulating symmetrically about its base. A, type of motion when phase difference between top and bottom of fin is 240°; B, when it is only 90°. The position of the fin base is indicated as a shaded oval area. Three successive positions of the fin border are indicated respectively in the full line, dashed line, and dash-dot line, and the directions of motion of the elements of the fin in each position are indicated by the small arrows. The fin rays are indicated only in the first position of the fin. It will be seen that in A the fin appears as an undulating membrane, while in B it appears to oscillate more or less as a plane.

It is frequently found that these pectoral fin movements may produce forward or backward locomotor forces, which may propel the fish at a considerable speed. This type of locomotion is very characteristic of the Labridæ. *Lachnolaimus* and *Thalassoma* swim almost entirely by means of their pectoral fins, as do all the angel fishes and the Scaridæ (parrot fishes). How can a longitudinal force be produced by undulatory fin movement of the pectoral fin?

One of the methods by which this undulation can be made to produce a forward- or backward-directed force is found in groups where the phase difference between the fin rays is very large, namely the Plectognathi. The fin ray is oscillated about a mean position which is inclined forward or backward to the direction indicated in figure 4. There is then a force component directed outward along this mean position of the fin ray, which will communicate to the fish a backward or forward motion. See figure 5, and also Breder and Harris, 1935. *Lactophrys* is a particularly capable exponent of this type of motion, using its pectoral fins for almost all its movements; the dorsal and anal fins are relatively small and less powerful than in other plectognath types such as the balistids.

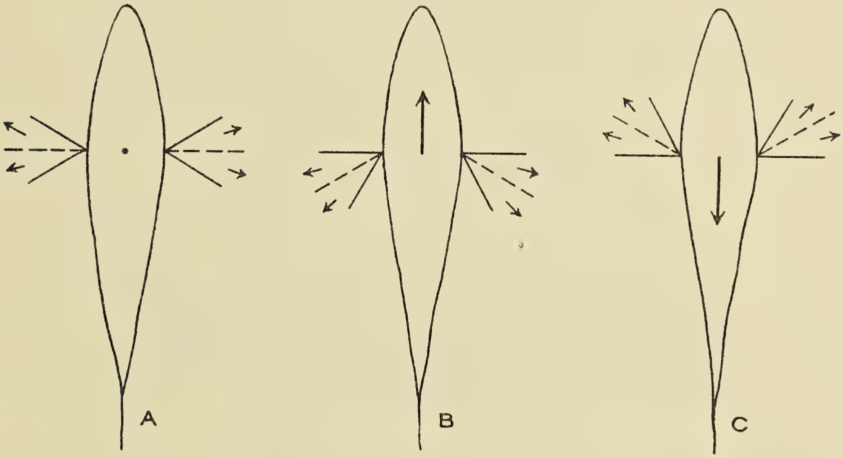

FIG. 5—A method of producing forward or backward locomotion by movement of the pectoral fins. The dotted lines indicate the mean position about which each fin ray is made to oscillate; the heavy lines represent the extremes of the oscillation of the ray. Small arrows show the direction of the forces produced by the oscillation, and the large arrow at the center of the fish body indicates the direction of locomotion produced. A, "neutral" position; B, forward locomotion; C, backward locomotion.

The currents produced by the undulation of the median fins of the plectognaths illustrate the presence of this "radial" component in a very striking manner. It will be noticed that in almost all the Balistidæ, the median fin base is not horizontal, but is inclined toward the tail. The dorsal fin base therefore slopes downward and the anal fin base upward. But cinematographic records have shown that the currents produced by these inclined fins are parallel to the long axis of the fish, and not to the sloping direction of the fin base. This is due to the combination of the two force components produced by the fin undulation: a longitudinal one which is parallel to the fin base, and a radial one which is perpendicular to this direction. The system is illustrated in *Monacanthus hispidus* in figure 6. It follows that the efficiency of these fins for locomotion is of a very high order: the resultant force which they exert is strictly parallel to the direction of motion which they produce.

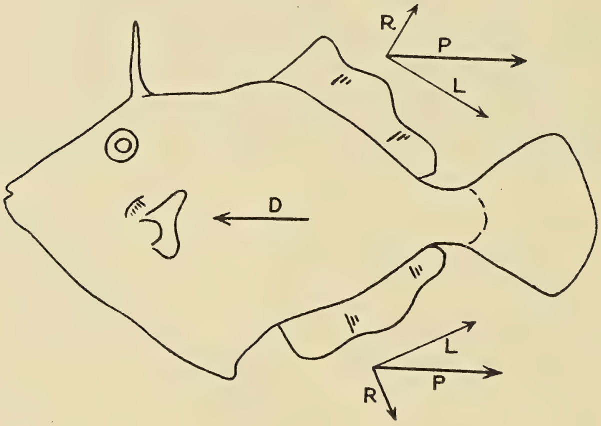

Fig. 6—The forces produced by the inclined median fins of *Monacanthus hispidus*. **R**, **L**, and **P**: the radial, longitudinal, and resultant forces produced by the dorsal fin (above) and the anal fin (below). **D**, arrow showing direction of motion of the fish, which is parallel to the direction of the two resultants **P**.

In percoid forms like *Epinephelus*, where the phase difference between successive fin rays is much smaller, another mechanism is incorporated in order to produce forward or backward locomotion. The "mean fin position" may be inclined forward or backward, as in the Plectognathi, but in addition the oscillation of any single fin ray is not symmetrical: the speed of the ray and the phase difference between the ray and its neighbor vary in different parts of the fin beat. This produces a definite asymmetry in the form of the beat in the two directions, and a large longitudinal locomotor force can thus be produced. Such a difference in speed of motion in the two directions can obviously be brought about by a difference in the relative force of the abductor and adductor contractions, while an asymmetry of phase relationships of successive fin rays can be produced by varying the strength of the muscular contraction to the different *segments* of the fin. Figure 7 illustrates the type of fin beat which would be produced if the phase difference between the fin rays were three times as great during one half of the fin stroke as in the other half. The horizontal force on the water

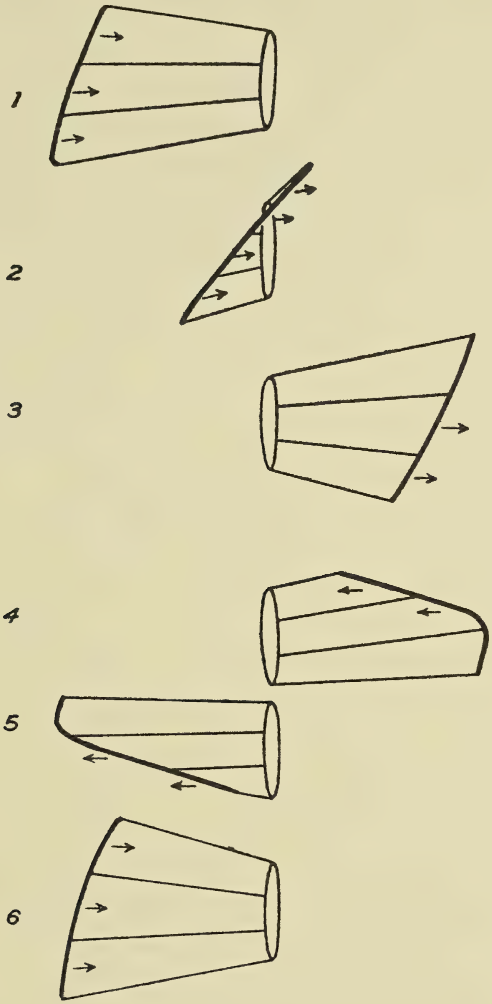

Fig. 7—Illustrating the production of a horizontal locomotor force by varying the phase difference of successive fin rays in different parts of the pectoral fin stroke. The fin is seen in side view, and six successive fin positions are shown. The oval area represents the fin base; a heavy line marks the fin border, and the light lines indicate the position of the fin rays. Small arrows show the direction of motion of the fin elements at any instant. The phase difference between successive fin rays is 15° during motion from left to right, 90° when moving from right to left. The greatly inclined position of the fin on the return stroke indicates that the resultant force produced will be from left to right.

in the one direction is obviously much greater than that in the opposite direction.

Since this difference in speed of motion of a fin ray is brought about by a variation in the intensity of the contractile force of the muscles attached to it, the size of the abductor muscles relative to that of the adductors is likely to vary in fishes which use their pectoral fins for different purposes. This is actually found to be the case. The ratio of abductor to adductor musculature varies from 0.65 to 2.3 in a number of forms examined by the writer. In the Scorpænidæ and Labridæ, which use their fins to a large extent for forward locomotion, but which may also use them for backing and hovering movements, this ratio is about 0.65, whereas in the Scombridæ, which use their fins as brakes, the ratio is 2.3. The Serranidæ, which use their fins chiefly for hovering and backing, but occasionally for forward locomotion, occupy an intermediate position. Since the size of these muscle groups can be correlated with their area of origin on the pectoral girdle, it is possible by an examination of the anatomy of the girdle to determine the type of fin motion which is characteristic of the animal.

The strength of the muscular contraction to the different segments of the fin can of course be altered by varying the motor nerve impulse so as to bring more or fewer muscle fibers into play. (This, of course, is also true for the strength of the abduction in relation to the adductor force of the fin.) But it is more efficient, as in the abductor-adductor relationship, to produce this result by an actual variation in the amount of musculature attached to the different fin rays. The presence of a well-marked adductor radialis muscle on the inner (posterior) side of the girdle in many percoids can be explained as an adaptation to the purpose of hovering and backing movements. In such backing movements, as has already been suggested, the fin undulation passes from bottom to top of the fin. Though the lower fin rays commence the abduction, the upper ones, supplied by their powerful muscles, rapidly overtake the lower ones, and thus the fin is moved forward as a more or less vertical surface, exerting a tremendous thrust on the water. But in the return stroke, the lowest adductor muscle, contracting first, is aided by the contraction of the adductor radialis. The lower border of the fin is adducted so rapidly that it is almost back to the side of the fish before the upper part has started to return, so that the fin plane is almost horizontal on the backward stroke, and the thrust on the water is small. That this is the probable explanation of the function of the adductor radialis is suggested by the fact that this muscle is negligibly small or absent (a) in forms like *Scorpæna* which use their pectoral fins for forward swimming, but do not hover or back with them to any extent, and (b) in forms like *Euthynnus* (Scombridæ) where the pectoral fins are thrown out as brakes, and are not used for *producing* motion.

An interesting method of producing a backward-directed locomotor force by pectoral fin movement is found in the chætodont fishes, of which *Angelichthys ciliaris* and *Pomacanthus arcuatus* have been studied in some detail by the writer. Here the upper fin rays oscillate about a mean position which is almost perpendicular to the long axis of the body, while the lowest fin rays oscillate about a mean position which is directed backward. The maximum possible amplitude of the lower fin rays is thus reduced, but the fin membrane as a whole is inclined backward at its lower end during motion.

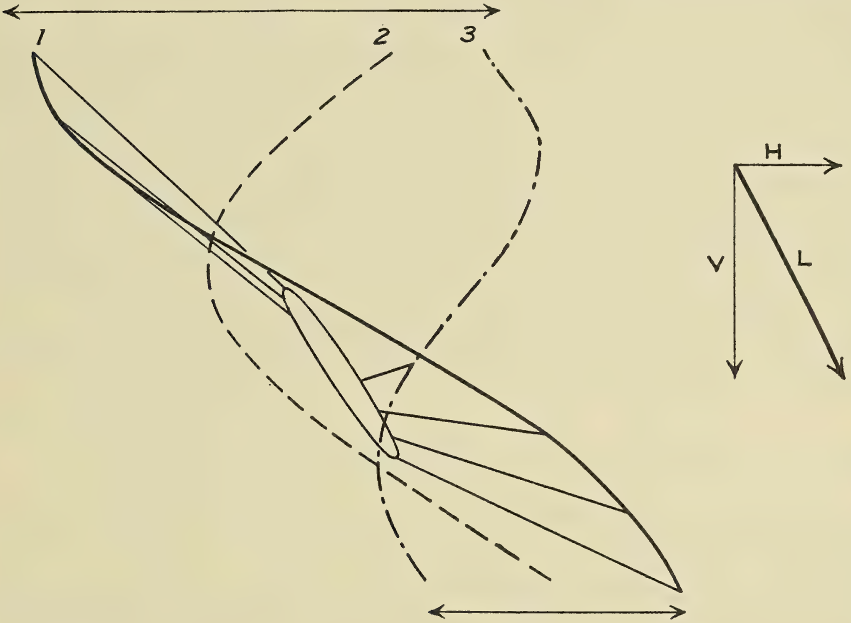

FIG. 8—Illustrating another method of producing a locomotor force by means of the pectoral fin. The fin base is represented by the oval in the center of the diagram; as in figure 4, three successive positions of the fin border are shown. The arrows above and below the fin represent the limits of oscillation of the upper and lower fin rays. It will be seen that the fin undulates about a mean inclined position. At the side of the diagram the "longitudinal" force (produced by the passage of the undulation along the fin) is represented at **L**; it can be resolved into a vertical component **V** and a horizontal component **H**, the latter being used to produce locomotion of the fish.

The "longitudinal" component (due to the undulation passing from top to bottom of the fin) is thus directed backward, instead of being vertical as in figure 4 (Plectognathi), and this component becomes of considerable importance in propelling the fish. This type of pectoral fin motion is illustrated in figure 8.

It is obvious that in any teleost fish the actual motion of the pectoral fin may be compounded of a number of these different types of movement, so as to give the most efficient resultant force needed for the purpose required. Probably there is no instance where one, and only one, of the above methods is found, to the exclusion of all others. The grouper, *Epinephelus,* uses a movement which is of a type similar to that of the chætodont form described above, when it is producing forward motion, but this is combined with a definite asymmetry of fin ray beat, so that the phase difference between rays is not the same at different parts of the fin stroke (as in fig. 7). Rolling movements in the same fish are produced by a fairly simple symmetrical oscillation of the fin rays, the undulation passing downward in the one fin and upward in the other, giving a maximum rolling couple with little translational force. In a single fish, therefore, not only may the actual motion of the fin be represented as a combination of several of the above types of motion, but the extent to which the different types are combined can apparently be varied to suit the particular requirements of the moment.

The only way to determine the actual motion of a fin is to make a cinematographic record of it, and to analyze the individual motions of the fin rays. This has been done for a number of different fish, but the presentation of the results here would take a great deal of space. It was therefore thought better to give an account here of the *possibilities* which have been established as a result of these photographs, since these are of much more value to the general student than the knowledge of each individual variation.

SUMMARY

1. The present paper discusses the mechanical factors concerned in the evolution of the teleost type of fish.

2. A comparison of this type with the dogfish suggests that the development of an air bladder has been the primary factor involved in the change in general body form.

3. The reduction in specific gravity of the fish, consequent upon this primary change, has removed the need for a lift force on the body during free swimming. The asymmetrical (heterocercal) tail has therefore disappeared.

4. For the same reason, the pectoral fins are no longer needed as elevating planes, and become free to move up toward the mid line of the body to act as brakes in stopping and turning movements.

5. The forward motion of the pelvic fins is a mechanism for producing a balanced vertical force and a balanced pitching moment. These fins are normally used in conjunction with the pectorals.

6. The independent movements of the pectoral fins are then discussed. All types of movement so far observed are variations on a fundamental form, in which the metachronal oscillation of the fin rays generates an undulating fin surface.

7. The observed variations in form of the fin beat can be produced by varying the phase difference between the beat of successive rays, and also by making the oscillation of the fin ray asymmetrical.

8. The characteristics of the pectoral musculature associated with such variations are pointed out, and illustrated by reference to a number of fish types.

In a general paper such as the present one it is impossible to acknowledge the assistance of more than a few of the people who have indirectly helped to produce it. To the Carnegie Institution of Washington and the Commonwealth Fund I owe the financial assistance which has made much of the work possible. To the late Dr. W. H. Longley I am indebted for the suggestion and identification of many of the types studied, and also for his keen and helpful interest in the problem during my two periods of residence at Tortugas. A part of this work was also carried out at the New York Aquarium, and I have to thank Mr. C. M. Breder and the staff of that institution for their very willing and capable cooperation.

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PAPERS FROM TORTUGAS LABORATORY, VOL. XXXI

VIII

STUDIES ON THE ABSORPTION AND SCATTERING OF SOLAR RADIATION BY THE SEA

Spectrographic and Photoelectric Measurements

By Hugh H. Darby, E. R. Fenimore Johnson, and George W. Barnes

With one plate and five text-figures

[Issued October 15, 1937]

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STUDIES ON THE ABSORPTION AND SCATTERING OF SOLAR RADIATION BY THE SEA

Spectrographic and Photoelectric Measurements

INTRODUCTION

A considerable amount of work has been done in recent times on the penetration of radiation into sea and lake water. The importance of this work, in such matters as plant and animal metabolism and under-water photography, is obvious. The amount and spectral distribution of scattering, and the penetration of the ultraviolet component, are two phases of the subject which have received but scant attention. It is with these subjects that this contribution will deal.

The studies were made from the yacht *Elsie Fenimore*, and at the Tortugas Laboratory of the Carnegie Institution of Washington. We wish to acknowledge the assistance of the late Dr. W. H. Longley, former Executive Officer of the Laboratory.

In general, two types of attack have been made on the problem: (a) a laboratory study, under controlled conditions, of the transparency of distilled and sea water for various wave lengths throughout the spectrum; (b) a study in the field, with the sun as a source, and under the varying conditions of humidity, agitation of the surface, salinity, sedimentation, and organic matter. Owing to the fact that Sawyer (1931) has reviewed the literature dealing with the former phase and Atkins (1932) has done the same for the latter phase, only pertinent literature will be referred to hereafter. The agreement among laboratory workers for distilled water is quite good. For sea water, however, results are highly variable.

Monochromatic radiation passing through water suffers an exponential diminution in intensity which may be expressed by the law

$$I = I_0 e^{-k_1 L} \tag{1}$$

where I_0 is the intensity at the beginning of a path of length L, I the intensity at the end of the path, and k_1 the transmissive exponent. This gives the total reduction in intensity, and is the sum of the loss through scattering and through absorption.

It is customary to use this relation in both field and laboratory measurements. The experimental work consists in evaluating k_1 for various wave lengths and under various conditions.

FIELD METHODS

The earliest methods were optical, and the subjectivity of the results soon led to the adoption of more precise methods. Recent determinations have, with few exceptions, been made with the photoelectric cell. This method of experiment possesses great advantages for field work. The apparatus is compact, simple, and relatively inexpensive. Most important of all, the current is nearly proportional to the amount of radiation incident on the cell, and the measuring instrument can be calibrated directly in terms of intensity.

These extremely valuable devices, however, do have certain limitations which affect their usefulness for submarine measurements. These limitations have been realized and discussed by workers who have used them (Clarke, 1933; Utterback, 1934). In our opinion, the most serious of these disadvantages is their unequal spectral response. Figure 1 gives the spectral sensitivity of the cells used by us. Other cells differ in the wave length to which they are most sensitive, and also in the breadth of their sensitivity curve, but this is a representative example. In many other studies, no attempt has been made to correct for the spectral sensitivity of the cells. Measurements taken with such an instrument are subject to extremely large errors, since a given change in current might be the result of a very small change in the component of the total radiation to which the cell is most sensitive, or a relatively large change in the components to which it is less sensitive. Furthermore, no information is obtained concerning the spectral distribution of the unabsorbed light.

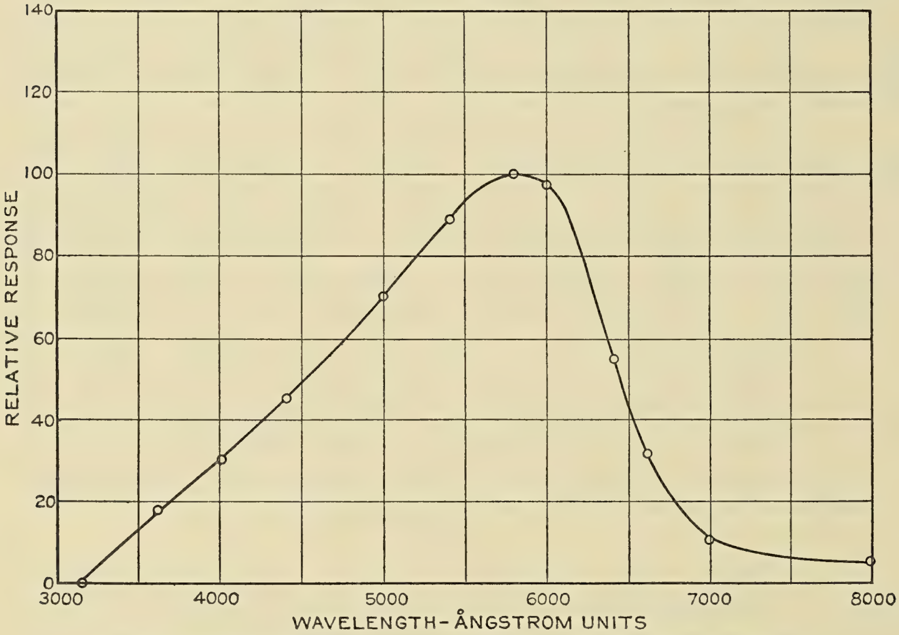

FIG. 1—Spectral sensitivity of photocells used in this investigation.

Increased accuracy is obtained by using several cells (Oster, 1935) having relatively narrow sensitivity curves, each covering a different part of the spectrum, or by isolating narrow bands of the spectrum with color filters (Utterback, 1933, 1934). This system gives intensity values for several regions of the spectrum. The radiation, however, is by no means monochromatic. This method is, therefore, subject to the errors discussed above, though to a lesser degree. Utterback and Jorgensen (1934) point out that this error is particularly serious in the red-orange and violet–near ultraviolet regions, where the transmissive exponent varies greatly throughout the region to which the cell or cell and filter combination is sensitive.

The spectrograph is valuable for under-sea research in that its indications are for each wave length of the spectrum, rather than for wide bands. Also, this instrument makes it possible to detect absorption bands, if present. It too has difficulties: all those peculiar to field work in this subject, and in addition, if a photographic method is adopted, those fundamental to photographic spectrophotometry. A very complete discussion of the latter is given by Harrison (1929).

The first recorded use of the spectrograph under water was that of Bertel in 1911, at Monaco. He used quartz optical parts and panchromatic plates. The shutter was opened and closed by messengers. It was necessary to bring the spectrograph to the surface to shift the plate.

Bertel obtained spectra to a depth of 500 m. At 600 m. he failed to obtain a trace, after a two-hour exposure. He found that the spectrum became narrower with increasing depth, and measured the shortest and longest wave lengths detectable on each spectrum. Figure 2 shows the limits obtained by him, together with the exposure and slit width at each depth. From these data, with no indication of the relative densities of the intervening portions of the spectra, or the sensitivity of the plates used, it is impossible even to estimate the transmissive exponent.

Bertel's research by no means realized the possibilities of the spectrographic method, but is stressed here for the following reasons: First, the work is of considerable historical importance, and seems to have been neglected in the bibliographies of the subject. Second, it is the only other observation comparable to Beebe's (1930) from the bathysphere (see also Hulbert, 1932). At 800 feet, Beebe reported a narrow band at about 5200 Å, fading out toward the violet. This wave length falls outside the band extending from 5000 Å to 3900 Å, observed by Bertel at the same depth. Since Beebe used a hand spectroscope with small dispersion, his wave-length measurement might easily be in error by the 200 Å, or more, necessary to bring these observations into agreement. Third, Bertel's work indicates a greater penetration of the near ultraviolet into the sea than would be expected from laboratory experiments. Since this is also indicated by our work, it will be discussed later.

Erikson (1933) used a photographic spectrophotometric method in his extremely careful study of the penetration of radiation into Gunflint Lake, in Minnesota. This water showed a maximum transparency at about 5800 Å, which is in disagreement with all laboratory and field sea-water measurements. The disagreement is no doubt caused by a difference in the impurities or dyes contained in the two types of water.

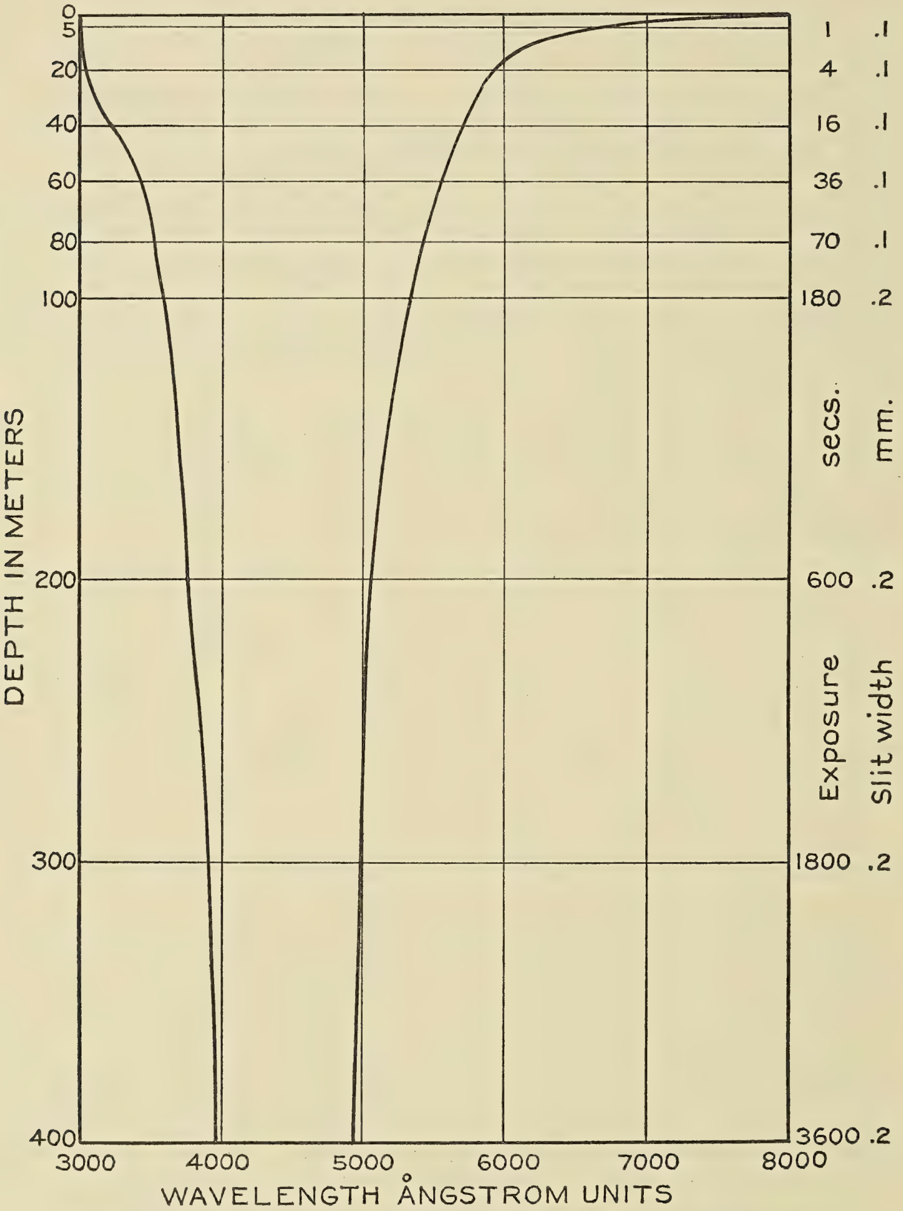

Fig. 2—Range of spectrum penetrating sea water at various depths with time of exposure and slit width, according to Bertel.

APPARATUS

THE PHOTOMETERS

Two Weston dry-disk type photoelectric meters were used. One was a standard three-range one-cell instrument, equipped with a 32× neutral density Wratten filter, and was used for determining the intensity of the light at the surface. The other was a standard two-cell three-range instrument. The cell member of this instrument was enclosed in a specially built watertight aluminum housing, with 78× Wratten neutral density filters, and was connected by a rubber-covered cable to the microammeter, which remained on deck. The cell housing was mounted upon a rigid tripod which permitted rotation of the instrument in both vertical and horizontal planes. These instruments were calibrated against a standard lamp, and were compared from time to time.

The photometers were used during the first season in a preliminary survey, and the following year in conjunction with the spectrograph, as a check upon the constancy of surface light and as a guide to the exposures with the spectrograph. No attempt was made to compensate these instruments for their unequal spectral response, shown in figure 1, nor to isolate spectral bands with color filters.

THE QUARTZ SPECTROGRAPH

The optical system was of conventional design, consisting of a window, micrometer slit, shutter, diaphragms, collimator lens, Cornu 60° prism, camera lens, and photographic plate. The shutter consisted of a single metal leaf, sliding in a plane parallel to that of the slit jaws, and with its edge parallel to the slit, thereby exposing and obscuring the whole height of the slit at the same instant. It was actuated by an electromagnet.

The plates used were Eastman type 1-L. These were 4 by 5 inches and permitted ten spectra to be taken on a single plate. The plate was shifted between exposures by a small electric motor, operating through a reciprocating motion and pawl, upon a ratchet rack attached to the plate-holder carriage. The electrical circuit included a locking relay to prevent the plate's moving more than one space, however long the shifting switch was kept closed.

A three-conductor rubber-covered cable connected the shutter and plate mover to the control box on deck. The power was furnished by a 9-volt dry-cell battery.

The spectrograph was mounted in a cast aluminum housing which served the double purpose of furnishing a rigid support for the optical parts and a watertight case for the instrument. The tripod mount incorporated leveling adjustments and two graduated dials permitting alignment of the spectrograph in any predetermined direction. In use, the instrument was set up and aligned by a diver, while exposures were made from the deck.

Since the spectrograph was set up on the ocean bottom, the depth of water was limited by the location. It was necessary to move the apparatus, and in most cases the launch, in order to obtain spectra at different depths. This involved a relatively long delay, during which the conditions of the water and radiation could vary. This uncertainty prevented the accurate determination of the transmissive exponent. In addition, the depth to which measurements could be extended was limited—in this case, to the 10-meter safety limit of the diving helmet. The practice of aligning the instrument by hand was adopted in order to maintain a narrow angle of view of the spectrograph, namely about 5°. This was important in order to obtain accurate directional measurements. The narrow angle of view, in turn, demanded a rigid support and careful alignment in order to bring the sun within the field for measurements of the direct radiation.

On shipboard the plates were reversed in the plateholder, and a series of calibration spectra were taken (see Plate 1A). The space available on the plate generally limited the number of these to three for each exposure time, which reduced the accuracy. The source was a 64-c.p. headlight bulb, and the effective intensity was reduced by means of an adjustable sector disk, rotating at a speed sufficient to minimize the intermittency error (Harrison, 1929). Density measurements were made with a recording densitometer, using a vacuum photoelectric cell. Characteristic curves for the plate were then plotted for a number of wave lengths throughout the spectrum, using the measured densities of the calibration spectra and the sector factors. Intensity values for the experimental spectra could then be obtained by interpolation from these curves.

STUDIES OF SCATTERING

During the summer of 1934, using the submarine photometer, a survey was made of the angular distribution of radiation at various depths under water. The sensitivity curve of this instrument is reproduced in figure 1. No attempt was made to correct its unequal spectral response, nor to isolate spectral bands with color filters. The results are therefore subject to errors for this reason. The "angle of view" of the instrument was about 97°. The reading at any given angle is not a true value for that angle, since the instrument was sensitive to light from a 97° cone, whose axis was the given angle. Because of these errors, the data may not be interpreted quantitatively, but much may be learned of the distribution by the trends of the curves. Figure 3 gives the results of a series of observations in which the instrument was swung from east to west, taking readings every 22.5°. The shift of the maximum is accounted for by the change in the elevation and azimuth of the sun. All the values have been corrected for the average surface intensity of 8800 foot-candles.

The point to be noted is that with increasing depth the vertical component is reduced to a much greater extent than the horizontal, which shows that the light was rapidly becoming more diffuse. This same fact was shown by Williams (1929) in the relatively more opaque waters of Puget Sound. He made a study of the horizontal and vertically upward intensity, and concluded:

> "Thus it is seen that for the direction in which the intensity is greatest to begin with, vertically down, the per cent absorbed by each meter is also the greatest; while in the direction in which the original intensity is the least, vertically up, the per cent absorbed per meter is also the least. Thus, as the depth increases, if conditions remained the same as they do for the first few meters, the absolute intensity from every direction would tend to become equal. That is, at great depths the light would seem to come equally from all directions."

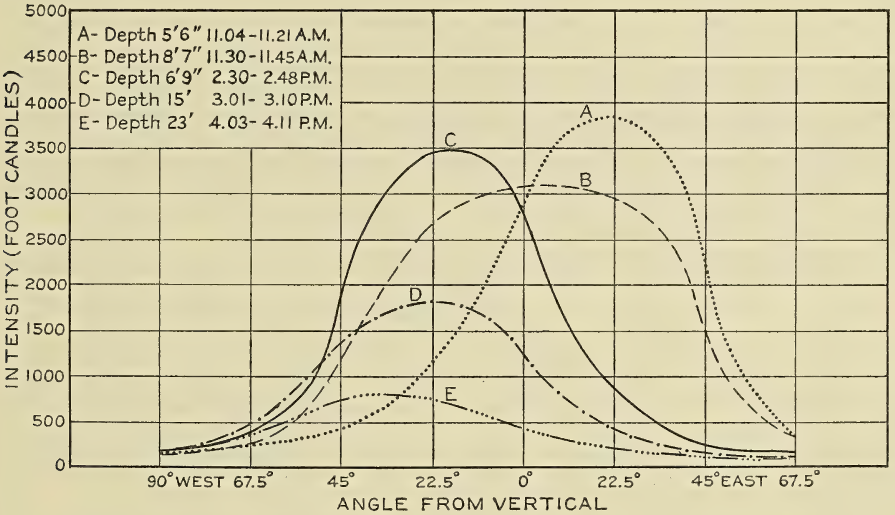

FIG. 3—Light measurement at Tortugas, July 21, 1934. Values corrected for average surface intensity of 8800 foot-candles.

A further study of scattered light was made in 1935, with the spectrograph, at several depths under water. All spectra were taken on days when the sky was clear, but not necessarily cloudless, and at times when the sun was unobscured. The constancy was checked by the deck and under-water photometers. Spectra were taken of the sun and the horizontally scattered light.

Values for the intensities in both spectra at several wave lengths were determined by interpolation from the characteristic curves.

Figure 4 gives the intensity values for a depth of 2.2 m. The curves are representative of those obtained at other depths. Curve A shows the intensities of the sun's spectrum, and curve B the intensities of the horizontal spectrum, plotted to an arbitrary scale. Curve C shows the horizontal intensities expressed as percentages of the solar. Curve C, then, shows the relative proportions of the incident light, of various wave lengths, which is scattered horizontally to a given point in the sea. This curve cannot be interpreted as an approximation to that of the spectral distribution of the scattering, since the scattered radiation is reduced in the water intervening between the point of scattering and the measuring instrument. The points on the curve B represent solutions of the equation

$$I = C\int_0^\infty I_s e^{-k_1 x}\,dx$$

where I is the measured intensity at a given wave length, I_s the intensity of that wave length scattered by unit volume, k_1 the transmissive exponent for that wave length, x the distance to the point of scattering, and C a constant involving the relative exposures of the two spectra and the angular distribution of the scattered radiation (see fig. 4).

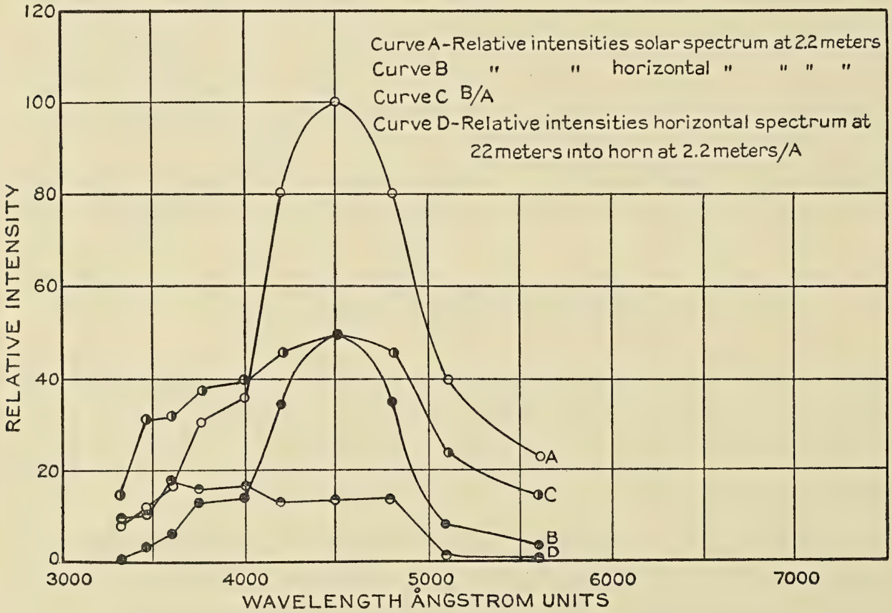

FIG. 4—Analysis of spectral transmission and scattering at a depth of 2.2 meters.

In order to determine from what distance the scattered light originated, a second spectrum was exposed on the same plate at the same depth. In this case, however, a light horn was placed 2.2 m. in front of the spectrograph.

This gave a spectrum of the radiation scattered in a horizontal column of water 2.2 m. long. This spectrum was compared with the direct solar spectrum in the same manner used for curve C. This procedure gave the curve D. A comparison of these two curves leads to the following conclusions:

1. Selective scattering was effective, the scattering increasing with decreasing wave length.
2. For short wave lengths the low values of curve C are accounted for by a strong scattering, reduced by re-scattering and absorption where the path from the point of scattering to the measuring instrument is long. In the case of the long wave lengths (>5000 Å) the primary cause of the low values is a weak scattering.

PENETRATION OF THE ULTRAVIOLET

In the course of our study of scattering, an unexpected amount of ultraviolet was observed in the solar spectra under water. Table 1 shows the penetration of ultraviolet light into sea water. The spectra from which the limits were obtained are reproduced in Plate 1B. The wave lengths given in table 1 are the shortest which were visible on the original plate, viewed under the most favorable conditions of lighting. In each case this limit is marked with a pair of dots. The trace here was not sufficiently dense to show in the reproductions; however, lines of only slightly longer wave length are clearly visible in the reproduced spectra. It is to be emphasized that the limits here given are not absolute, but are simply the shortest wave lengths of which a trace was found on our spectra of greatest density. These particular spectra are all somewhat overexposed in the visible region, but

TABLE 1—*Shortest wave lengths recorded at various depths, with summary of prevailing conditions*

(Slit width 0.02 mm. for all exposures)

Spectrum number	Depth (meters)	Shortest wave length recorded (± 2 Å)	Length of exposure (seconds)	Hour of day	Solar elevation	Photometer readings (foot-candles)	
						Surface	Under water
23-10...	0.00	3006	¼	10:30	61°12′	....	....
29-2 ...	0.33	3052	1	1:42	74°30′	9150	5005
20-3 ...	1.60	3006	4	2:40	60°20′	....	2695
19-6 ...	2.20	2972	10	12:30	88°24′	9920	3850
28-4 ...	2.26	2979	10	11:36	75°32′	9100	3465
22-1 ...	3.42	2997	6	11:15	71°29′	8998	3465
21-10...	3.42	3036	1	12:47	86°57′	8998	3542

only by a relatively small ratio. No doubt somewhat shorter wave lengths could be detected by prolonged exposures.

As previously stated, our method was not adapted to the extremely accurate measurement of the transmissive exponent. In order to obtain quantitative results, the following procedure was adopted. On the same day, exposures were made of the solar spectrum at two depths. In the first case, the layer of water over the spectrograph was 2.2 m., the time was about an hour before local apparent noon, and the sun's elevation was 75°05′. The

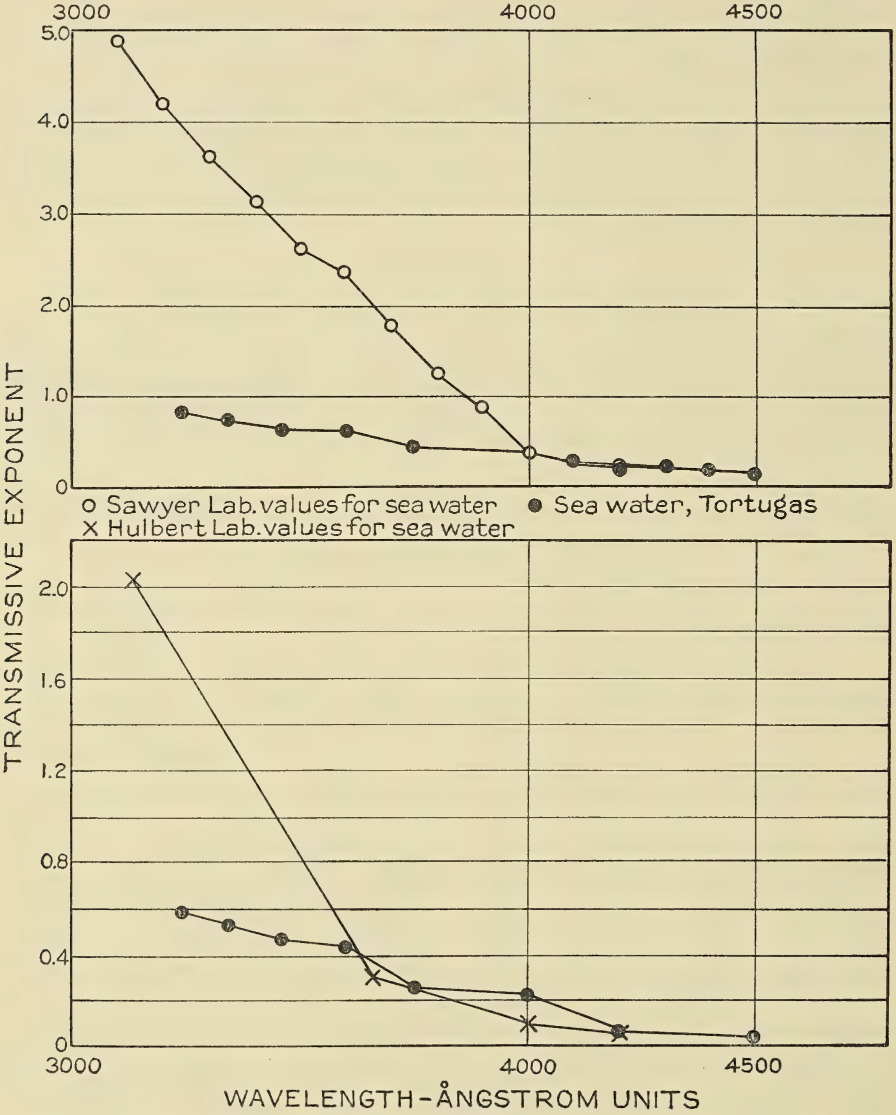

FIG. 5—Comparison of transmissive exponents obtained by Sawyer, Hulbert, and ourselves.

second exposure was made at a depth of 0.33 m., the time was about an hour after local apparent noon, and the sun's elevation was 74°30′. In each case the exposure was 1 second, and the slit width 0.02 mm. The surface intensity differed by 3 per cent, as measured by the deck photometer.

In the spectrum at 0.33 m., the intensity at 4500 Å was arbitrarily assigned a value of unity and the relative intensity determined at several shorter wave lengths. These are given as values of I_0 in column 2, table 2.

Values of I were obtained from equation (1),

$$I = I_0 e^{-k_1 L} \tag{1}$$

where I_0 was unity, L was 1.87, and k_1 was assigned the laboratory-determined values of 0.02 (Hulbert,[1] 1928) and 0.169 (Sawyer, 1931) for sea water at 4500 Å. Intensities of 0.965 and 0.725, respectively, were obtained. These values, in turn, were assigned to the intensity of the spectrum at 2.2 m., for 4500 Å, and the corresponding relative values for shorter wave lengths were determined from the plates. The resulting intensities are given in columns 3 and 4 of table 2. The values of the transmissive exponent were then calculated for the various wave lengths, using equation (1) with the values in column 1 as I_0 and those in columns 3 and 4 as I, and making L equal to 1.87. These exponents are given in columns 5 and 6, table 2, and are plotted together with the laboratory results in figure 5. The disagreement between field and laboratory results is seen to

TABLE 2—*Relative intensities of various wave lengths at two different depths, and the corresponding transmissive exponents of sea water, calculated on the basis of Hulbert's and Sawyer's values for k_1 at 4500 Å*

1 Wave length in Å	2 I_0 Relative intensity at 0.33 m.	3, 4 I Relative intensity at 2.2 m.		5, 6 k_1 Transmissive exponents calc. from our data	
		$k_1=0.02$	$k_1=0.169$	$k_1=0.02$	$k_1=0.169$
4500....	1.00	0.965	0.725	0.02	0.169
4200....	0.82	0.75	0.57	0.047	0.195
4000....	0.52	0.35	0.25	0.21	0.39
3750....	0.47	0.30	0.22	0.24	0.43
3600....	0.36	0.16	0.116	0.43	0.61
3460....	0.26	0.11	0.08	0.46	0.63
3340....	0.22	0.08	0.058	0.53	0.7
3240....	0.11	0.035	0.025	0.58	0.8

---

[1] Hulbert made no determination at this wave length. The value given represents a graphical interpolation between values at 4360 Å and 5460 Å.

be quite serious. The belief that this disagreement is real is supported by independent evidence of another type.

On our spectrum 22–1, exposed 6 seconds at a depth of 3.4 m., a trace at 3010 Å was visible. This was compared with the trace at the same wave length on the surface spectrum 23–10 exposed ¼ second. The exposure ratio (24:1) was not large enough to involve a great error due to the reciprocity law failure (Harrison, 1929). Using this exposure ratio and the relative densities, it was found that the surface intensity was reduced to about one thousandth by this depth (3.4 m.) of water. The lowest laboratory value for the transmissive exponent of sea water at this wave length, 3010 Å, is that of Hulbert (1928). Using this value of $k_1=1.7$ and $L=3.4$ in equation (1) would give a reduction in intensity to about one millionth of the surface value.

Bertel's results with the spectrograph are difficult to interpret, for the reasons given in our summary of his work; nevertheless the following points are to be observed in the graph of his results given in figure 2: (1) The ultraviolet penetrates to relatively great depths, as we have observed using the same type of instrument. (2) The shortening of the spectrum is much more abrupt and marked at the red end.

That much shorter wave lengths reach the earth than had been previously supposed has been shown recently by the use of Geiger-Müller photoelectric counters. Even in northern regions (New York City) definite counts have been recorded with a gold counter, which responds only to wave lengths of 2600 Å and shorter. Previous failures to record these wave lengths have been largely of instrumental origin, and have been due to absorption and scattering by optical systems and the insensitivity of the photographic plate to light of very low intensities. The present measurements, however, show that even within the spectrograph's range of sensitivity, shorter wave lengths are obtained in the summer in southern Florida than have been reported by other investigators.

A biological application of these findings has already been made. It is well known that the activation of ergosterol to calciferol is best accomplished by wave lengths in the vicinity of 2650 Å. It was clear from the data presented above that if any marine plants contain a vitamin D-like substance, produced by irradiation with these short wave lengths, the irradiation must occur close to the surface of the water. The sargassum weed, which grows in shallow water and later breaks loose to float on the top of the sea, was therefore examined from this viewpoint. The oils from this alga were found to possess marked antirachitic activity (Darby and Clarke, 1937).

The bearing of these observations on the ecology of tidal forms, both plant and animal, is obvious; and the difficulty of keeping certain marine organisms alive under laboratory conditions may well originate in unnatural, and probably inadequate, illumination.

SUMMARY

A comparative study has been made of two methods of evaluating the transmission of various wave lengths of light through sea water: (1) photometry by means of photoelectric cells, and (2) photographic spectrophotometry.

Bertel's observation that ultraviolet light penetrates a considerable distance into the sea has been confirmed. The extent of penetration is greater than would be expected from the laboratory data of Hulbert and Sawyer. A rough evaluation of the transmissive exponent from 4500 Å to 3250 Å was made, which indicates the magnitude of the disagreement.

Scattering was found to be selective, becoming greater with decreasing wave length. The spectral distribution of scattered radiation is indicated.

The importance of these observations for biological systems is outlined.

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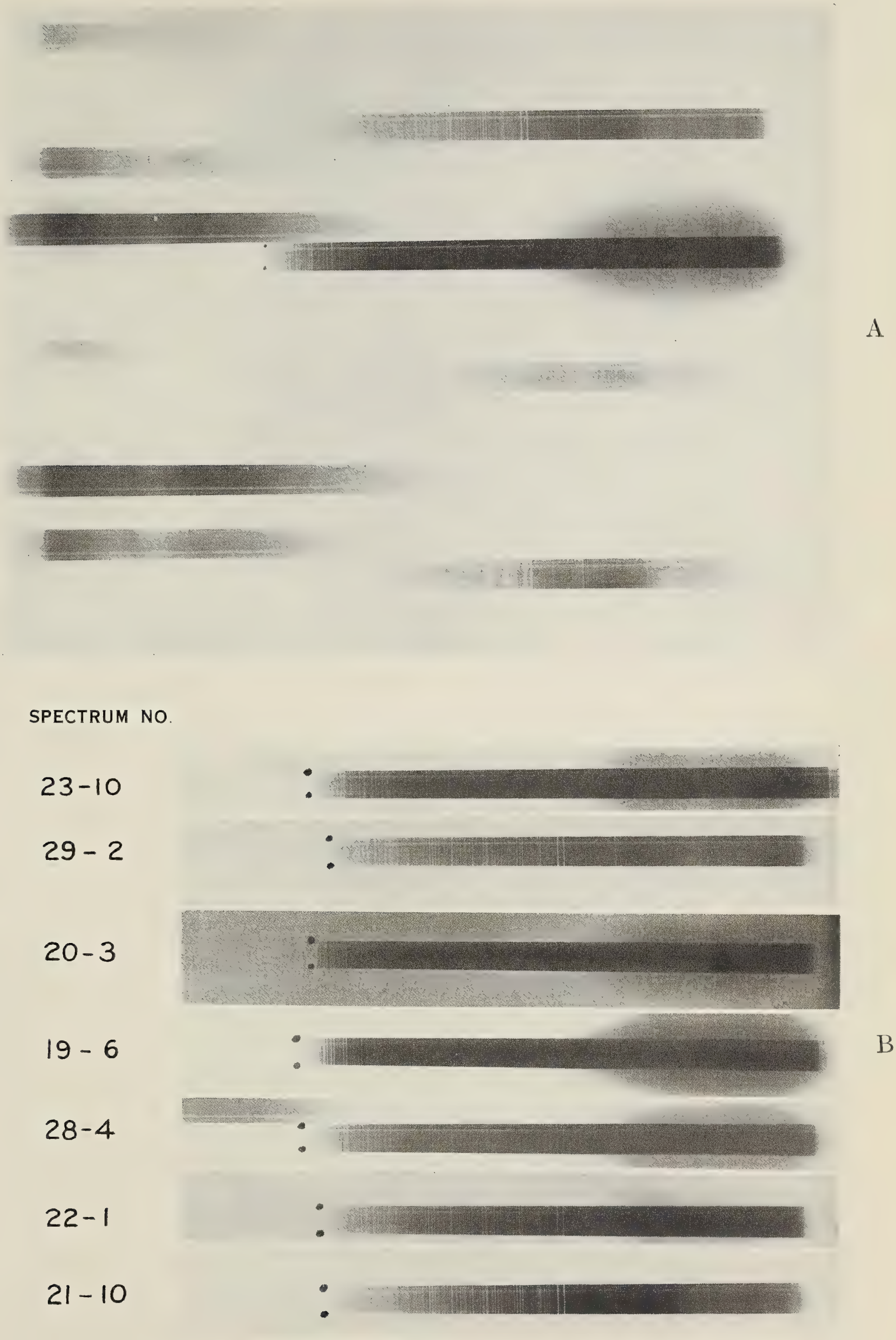

A. Sample plate showing field and calibration exposures.

B. Spectrograms on which table 1 is based.

PAPERS FROM TORTUGAS LABORATORY, VOL. XXXI

IX

STUDIES ON THE BIOLOGY OF TORTUGAS CORALS

III. The Effect of Mucus on Oxygen Consumption

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[Issued October 30, 1937]

STUDIES ON THE BIOLOGY OF TORTUGAS CORALS

III. The Effect of Mucus on Oxygen Consumption

In the course of his work on the toxic effects of high temperature on Tortugas corals Mayer (1918) found great differences between the relative oxygen consumption of different representative species. Later (Mayor, 1924) he carried out similar determinations on Pacific corals at Samoa and obtained somewhat less striking differences. His figures are given in table 1.

TABLE 1—*Relative oxygen consumption of (A) Tortugas corals, (B) Samoan corals, as determined by Mayor*

A		B	
Siderastrea radians	1.0	*Porites andrewsi*	1.0
Mœandra areolata	3.8	Massive *Porites* aff. *P. lutea*	2.0
Favia fragum	5.5	*Acropora formosa*	2.4
Orbicella annularis	6.1	*Pocillopora damicornis*	5.5
Acropora muricata	18.7		

Mayor found that in general the species with the highest apparent oxygen consumption were more affected by high temperatures than were those with low oxygen consumption. For instance, among Tortugas species, *Acropora muricata* was killed at 34.7°C. while *Siderastrea radians* was not killed until the temperature was raised to 38.2°. He considered that death from high temperatures might be the result of the accumulation of acid (possibly H_2CO_3) in the tissues and since this would accumulate most rapidly in the case of animals with higher metabolism they would be killed at the lowest temperatures. He overlooked one important factor when making this statement, namely the presence of the zooxanthellæ, which, as shown by Yonge and Nicholls (1931), utilize, in the light, all of the CO_2 produced by the animal. For this reason, therefore, it is difficult to accept his conclusions.

Nevertheless the extremely wide range in the metabolic rate of the different species of Madreporaria indicated by Mayor's figures clearly demands some explanation. The figures have been criticized by Verwey (1930, 1931) and by Vaughan (1930). The former (1931) points out that, according to Mayor, "*Acropora muricata* consumes per kilo of its living tissue per hour about 500 cc oxygen. Compared with the figures given for related animals—i.e. *Cassiopea* (26 cc), Anthozoa (40), Alcyonaria (living tissue only) (14-75)—this figure . . . is very high, especially if one realizes that we are dealing with sessile animals. Higher, fast moving animals of course show high figures: cephalopods and crustaceans 200 and more, fishes 200-500 cc, and more."

The wide variation in the content of zooxanthellæ in different species (Yonge, Yonge and Nicholls, 1932) suggested that it might be possible to correlate this with metabolic rate, i.e. the greater the content of zooxanthellæ the higher the metabolism of the coral owing to the more rapid removal of the waste products of metabolism. To determine this, experiments were carried out on a variety of coral colonies. Oxygen consumption, algal content (in terms of oxygen production in the light) and weight of living material, were all determined. It is sufficient to add that no such correlation was found.

Before the results of these experiments could be analyzed and, unfortunately, only a short time before the completion of the visit to the Tortugas Laboratory in 1934, an alternative explanation of the variations in metabolic rate became apparent. This was suggested by the fact that all species of *Acropora,* which are characterized by so high a consumption of oxygen, secrete great quantities of mucus. When taken out of water the branches literally drip with mucus. *Acropora* is very difficult to keep in captivity, apparently owing to the mucus which can be seen to collect around the branches in aquaria. It appeared also that there might be some correlation between this great production of mucus and the branching of the colonies. Cary (1918) found that in Alcyonaria from Tortugas the species which have the greatest surface per unit weight have by far the highest apparent metabolism. Incidentally, he was unable to establish in these animals any correlation between the temperature causing death and the rate of oxygen consumption.

Certain experiments, limited in number owing to lack of time, were set up to test the possible effect of mucus production on oxygen consumption. Selected corals, carefully cleaned at the broken edges where necessary to remove all decaying matter, were placed in large screw-top jars of capacity a little under three liters. These were filled under water and then left for a definite period under certain specified conditions. At the end of this period the jars were carefully shaken to remove any local differences in the oxygen content of the contained water and two samples of water were taken from each jar. The first samples were treated immediately for subsequent determination of oxygen content by Winkler's method, the second samples were left for definite periods and then treated. The purpose of this was to determine if and to what extent the oxygen content fell in water which had previously been in contact with the corals. Control experiments were run but in no case did the fall in oxygen exceed the experimental error of the method. Details of the first experiment are given in table 2.

These results show clearly that the oxygen content continues to fall after the water has ceased to be in contact with the corals and indicate that this may be due to the oxidation of the mucus which is exuded by the corals and passes into the water. The differences between the fall in the oxygen content of the two samples in the various corals used are of no significance because the corals varied in size.

A further experiment was run using *Acropora muricata* and *Mœandra areolata.* Specimens of the former were collected immediately before use so as to ensure that they were in good condition. The results are given in table 3.

TABLE 2—*Corals exposed to the light in a wire netting crate about ½ meter below the surface. Samples taken after 5 hours, sample A precipitated at once, sample B 5½ hours later.*

Species	Oxygen in cc. per liter		Difference
	Sample A	Sample B	
Mœandra areolata	10.67	10.49	—0.18
Porites asteroides	8.89	8.70	—0.19
Orbicella cavernosa	7.79	7.51	—0.28
Siderastrea radians	9.54	9.25	—0.29
Orbicella annularis	8.98	8.67	—0.31
Mœandra clivosa	8.95	8.42	—0.53
Acropora muricata	10.20	9.64	—0.56

TABLE 3—*Corals exposed to moderate light in the shade in the aquarium. Samples taken after 3 hours, sample A precipitated at once, sample B 6 hours later.*

Species	Oxygen in cc. per liter		Difference
	Sample A	Sample B	
Acropora muricata	2.79	2.25	—0.54
Acropora muricata	3.17	2.85	—0.32
Mœandra areolata	2.76	2.19	—0.57
Mœandra areolata	2.68	1.29	—1.39

This experiment is of interest because the *Mœandra* were collected at one of the planulating periods (see Yonge, 1935); both contained great numbers of planulæ and the second individual actually discharged great numbers of these during the course of the experiment. A great deal of mucus was extruded with them so that the water appeared cloudy. This is reflected in the very great fall in oxygen in sample B.

A final experiment was carried out using *Acropora muricata* and *Siderastrea radians,* the two species which gave most widely different figures in Mayor's experiments. In this case experiments were carried out in both light and darkness as shown in table 4.

This experiment indicates that the discharge of mucus is approximately the same in light as in darkness and consistently higher in the case of *Acropora.* Since the initial oxygen figure was 4.0 cc. per liter it follows that, in the dark experiments, there was, taking the average account of the four experiments, a fall of oxygen in *Acropora* of 1.37 cc. per liter and in *Siderastrea* of 0.875 cc. per liter. Assuming that the oxidation of mucus proceeded at the same speed during the experimental period as it did during the subsequent 5 hours (actually it would almost certainly be greater) we find that 34.3 per cent of the apparent respiration of *Acropora* is due to oxidation of mucus and only 14.3 per cent due to that cause in *Siderastrea.* In view of the immense amounts of mucus invariably produced by *Acropora* it is highly probable that in species of this genus the relation of actual to apparent respiration is much lower than indicated by the figures given above.

Table 4—*Corals exposed first in a light-tight box ½ meter below the surface of the sea and later in the light in the wire crate, in the same jars and in the same situation. In both cases experiments were run for 5 hours, sample A being precipitated at once and sample B 5 hours later. The average water temperature was 29.5°C in both experiments. Initial oxygen content of water in both cases 4.0 cc. per liter.*

Coral	Dark O_2 in cc. per liter			Light O_2 in cc. per liter		
	A	B	Diff.	A	B	Diff.
Acropora I	2.81	2.44	—0.37	8.66	8.34	—0.32
II	2.85	2.36	—0.49	9.56	9.03	—0.53
III	2.74	2.33	—0.41	8.85	8.52	—0.33
IV	2.11	1.50	—0.61	10.19	9.41	—0.78
Average difference			—0.47	Average difference		—0.49
Siderastrea I	3.14	2.96	—0.18	6.38	6.23	—0.15
II	3.08	2.99	—0.09	6.30	6.20	—0.10
III	3.06	2.98	—0.08	4.82	4.69	—0.13
IV	3.22	3.07	—0.15	6.92	6.77	—0.15
Average difference			0.125	Average difference		—0.133

The experimental data supplied, unfortunately but unavoidably scanty as they are, are sufficient to throw grave doubt on the validity of all figures hitherto published on the respiration rate of corals. Mayor's figures are inherently improbable—a difference of metabolic rate in animals of the same order and of the same sessile habit of up to 18.7 times cannot but be questioned—and the oxidation of mucus during the course of the experiment provides a reasonable explanation for these differences. Cary's figures for respiration in Alcyonaria can be accounted for in the same way because he explicitly states that respiration appeared greatest in species where there was the greatest surface—and hence possibility of mucus production—to volume of living tissue.

A further point arises in connection with Verwey's work on the significance of zooxanthellæ in controlling the depth to which coral reefs may extend. On the basis of experiments carried out with *Acropora hebes* he calculated that an ordinary colony of a large *Acropora,* weighing several kilograms, would consume during a tropical night 250 cc. of oxygen for every kilogram it weighs. He adds, "According to such a calculation a reef of some thousands of kilos consumes several hundreds of liters of oxygen during one night. And as we may say that the water around these reefs contains about 5 liters of oxygen per cubic meter we understand that such a reef is able to deprive about 120 cubic meters of wholly saturated water of all its oxygen." Verwey comes to the general conclusion that the oxygen produced by the zooxanthellæ during the day time (for figures see Verwey, 1931; Yonge, Yonge and Nicholls, 1931) is essential for the maintenance of this accumulation of living matter. The force of this argument, which has already been questioned on other grounds—namely that in the absence of zooxanthellæ the surrounding water would be enriched with the excrement of the corals and so permit of a greater development of phytoplankton which would equally produce oxygen in the day time—is vitally affected by the validity of Verwey's figures for oxygen consumption. It is particularly unfortunate that he should have used a species of *Acropora* in his experiments on oxygen consumption.

In conclusion attention should be drawn to the probability that this hitherto unsuspected factor of error may be expected to apply to figures for respiration obtained for *all* aquatic animals which normally produce mucus in large amounts, either for feeding or for cleansing purposes or for both. It is difficult to see how this source of error can be avoided or how adequate allowance can be made for it.

Acknowledgments are due to the Carnegie Institution of Washington and to the Royal Society of London for reasons recorded in the first paper of this series. The writer wishes also to record his deep personal appreciation of the help received from the late Dr. W. H. Longley.

SUMMARY

Experiments are described which indicate that a large proportion of the *apparent* utilization of oxygen by corals is actually due to oxidation of mucus secreted by them during the course of the experiment.

The amount of mucus varies greatly in different genera and may also be increased at certain times, e.g. during planulation.

In view of this source of error it is impossible to accept at their face value figures which claim to represent either the absolute or the comparative rates of respiration in different corals, or general conclusions which are based on these figures.

Oxidation of mucus may be expected to affect the apparent rate of respiration in all aquatic animals which normally secrete mucus.

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